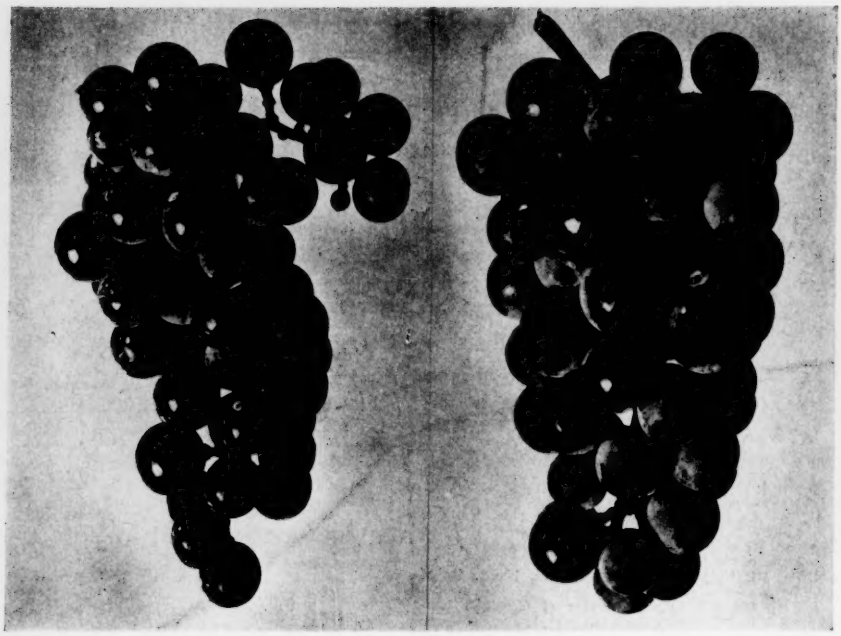
The Journal of HEREDITY



COLCHIPLOID GRAPES
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White Mink

Ragged Mice

"Hairy" Turkeys

Plato's Genetic Theory

Houseflies Resistant to DDT

Chicken-Pheasant Hybridization

VOLUME 45 JULY-AUGUST, 1954 NUMBER 4

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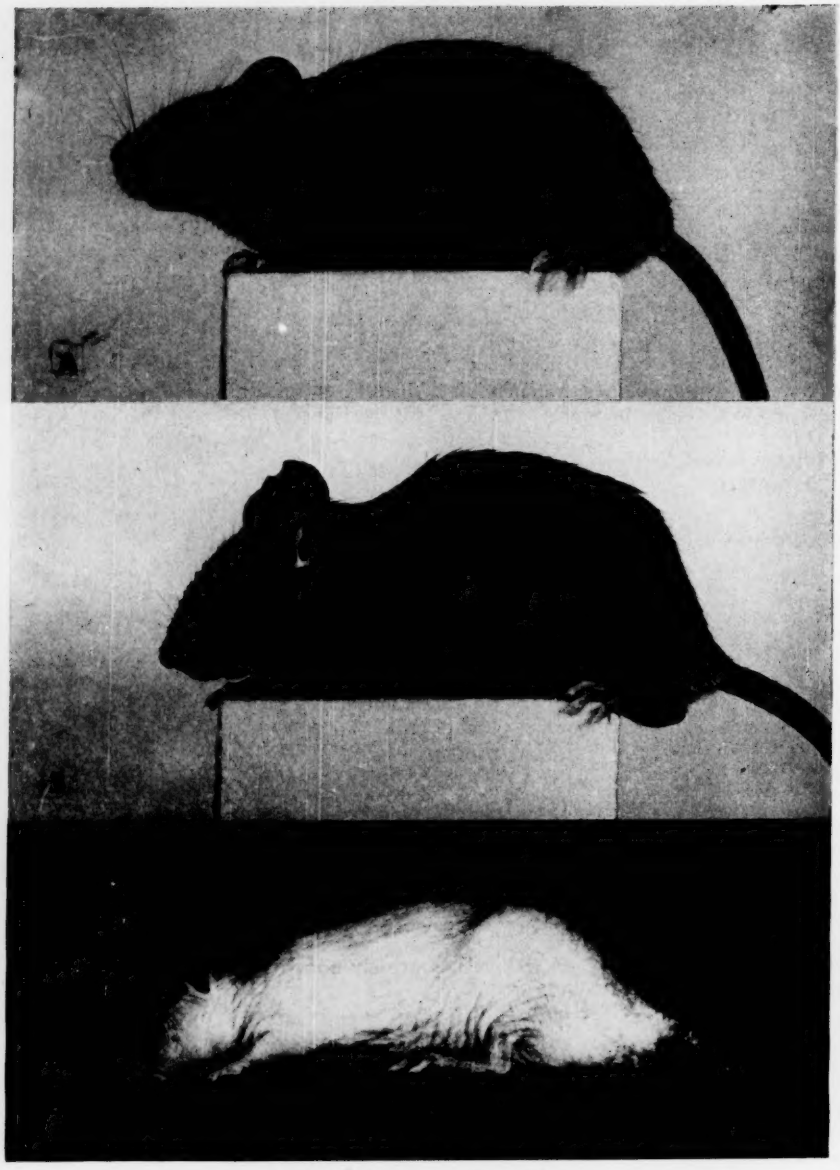


NORMAL AND RAGGED MICE

Figure 1

A—shows a normal mouse. *B*—is an adult heterozygous ragged mouse. Its appearance suggests that many of the hairs are absent. The guard hairs stand out from the coat more than in a normal mouse. *C*—shows a homozygous ragged mouse which is entirely naked except for a few pelage hairs confined to the posterior and ventral part of the body.

RAGGED, A SEMIDOMINANT COAT TEXTURE MUTANT

In the House Mouse

T. C. Carter and Rita J. S. Phillips*

THERE are three main classes of mutant genes in *Mus musculus* which affect coat texture: those which wave the coat (e.g. rex), those which remove it (e.g. naked) and those which change the relative numbers of the hair types (e.g. crinkled).

This paper describes a new semidominant mutant which belongs to the last class; its locus is at one end of linkage group V.

Origin

The first ragged mouse, a female, was one of a litter of ten sired in a test of her father's fertility; he was from a cross-bred stock in which a translocation had been induced five generations earlier by X-rays. The ragged phenotype subsequently proved to be due to a gene which is semidominant; it must, therefore, have arisen spontaneously, either in one of the gametes which gave rise to the mutant animal, or as a sectorial mutation in one of her parents' gonads. Her father, having been found not to carry the translocation, was killed; it was thus impossible to test him for a mosaic gonad after discovery of the ragged mouse. Her mother was test-mated to an unrelated wild-type male, but bore only eight more young; all were normal. The ragged female was mated to the same male and bore 47 young, of which 24 were ragged.

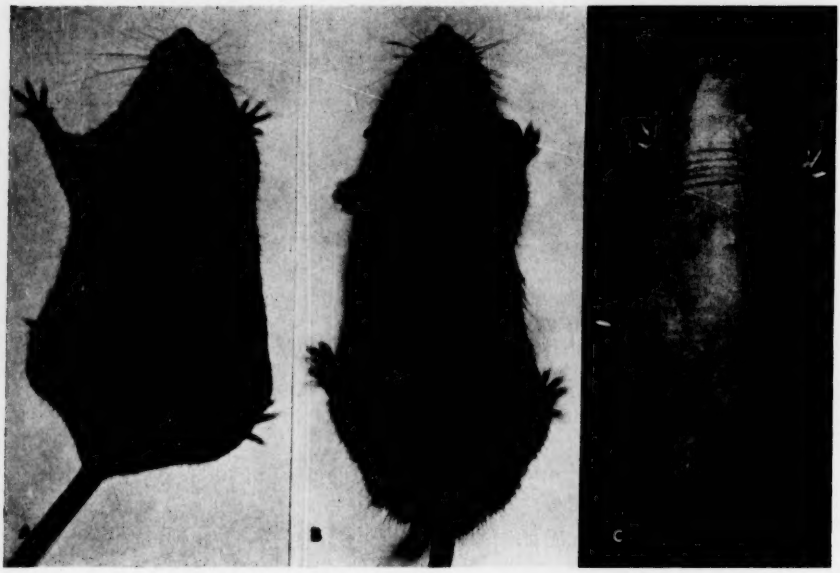
Ragged Heterozygotes

Heterozygotes can often be recognized at birth, in segregating litters, by their vibrissae, which are slightly shorter than normal; but this classification is not always reliable. Between two and four days, when the pigmentation of normal mice first becomes visible, heterozygotes color abnormally slowly. This can form the basis of a reliable classification, provided color mutants are not also segregating. Between three and six days down can be seen along the dorsum of a normal mouse when it is held silhouetted against a light; in heterozygotes the down is shorter, sparser, and develops about a day later. By nine days the coat of a heterozygote appears macroscopically different from that of a normal sib, in that it is obviously thinner. This difference remains throughout life.

The coat of the adult heterozygote (Figure 1*B*) looks sparse and lacks cohesion; its appearance suggests that many of the hairs are absent. There is no waving. Many guard-hairs are present, and they seem to stand out from the coat more than in the normal mouse —the feature which gave rise to the name 'ragged'. Pinches of hair from the mid-dorsal region include many guard-hairs, awls and auchenes, but the proportion of zigzags is much lower than in normal mice. Vibrissae are present and grossly normal. The distribution of the yellow agouti pattern is abnormal, the entire coat being unusually dark. This effect is strongest in the region of the dorsal midline, which may be completely black. The cheeks are affected least and, when color mutants are segregating, classification for agouti is most easily based on their appearance. Tail rings are present and the claws and eyelids appear normal.

The distinction between adult hetero-

*Institute of Animal Genetics, Edinburgh, Scotland; the authors are Members of the Scientific Staff, Medical Research Council. We are grateful to Miss M. M. Manson, who found the first ragged mouse, and to Miss G. I. E. Mayor for their technical assistance, and to Mr. D. W. M. Pinkney who took the photographs.



RAGGED HETEROZYGOTE AND HOMOZYGOTE

Figure 2

A—shows a normal house. *B*—is a heterozygous ragged mutant. The vibrissae are shorter than in normal and the hair is shorter and more sparse. *C*—shows a naked homozygous ragged mouse. There are a few sinus hairs present, but these are shorter than in the normal mouse.

zygotes and normal mice is always quite clear; there is no reason to suppose that normal overlapping may occur.

Heterozygotes may develop a little more slowly than their normal sibs, especially in large litters; but viability does not appear to be appreciably affected. Breeding performance is normal; segregation data are given in Table I.

Ragged Homozygotes

When ragged heterozygotes were mated together, they produced ragged and normal young in a ratio of 2:1 (Table I), suggesting that there are no homozygotes in the ragged class. In addition these matings gave some abnormal young which formed a homogeneous group, characterized at birth by the non-eruption of all but a few sinus hairs; the few which did erupt were the most posterior of the moustache hairs, and were very short. Many of the abnormal young also had a grotesquely blown-up appearance, due to generalized oedema; most of these died shortly after birth. Survivors grew slowly, but some became adult and were proven by breeding tests to be *RaRa* homozygotes. Adult homozygotes (Figure 1*C*) are naked, their few isolated pelage hairs being confined to the posterior and ventral part of the body. Sinus hairs are few and may be short; the moustache is represented only by the two or three posterior hairs in each row; the postorbital sinus hair is present, but the postorals are absent and the supraorbitals reduced from two to one on each side. The ears and tail are

TABLE I. Segregation of *Ra*

Genotypes of parents	Phenotypes of progeny		
	RaRa	*Ra*	+
Ra+ × ++	0	388	374
Ra+ × *Ra*+	21*	81	44
RaRa × ++	0	31	0
RaRa × *Ra*+	19	26	0

*Some of fourteen unclassified stillborn may have been *RaRa* homozygotes.

pigmented. Tail rings are present and there are no kinks in the tail. The claws appear normal.

There is nothing to suggest any overlap of the homozygous and heterozygous phenotypes; *Ra* is thus incompletely dominant.

The intercross segregation data of Table I show that the abnormal group could only account for about one-half of the expected homozygotes. However, there were also some stillborn young, many of which were partly eaten and unclassifiable; some may have been homozygotes. Other homozygotes could have died and been eaten before examination of the litters. Thus there is no conclusive evidence of prenatal mortality of homozygotes.

Linkage Tests

Ragged mice were outcrossed to standard linkage-testing stocks (Carter and Falconer[2]) and multiple heterozygotes from the F_1 generation were back-crossed. This provided segregation data testing *Ra* for linkage with markers in all the known linkage groups except group IV. Statistically significant evidence was found of linkage with the closely-linked marker genes short-ear, *se*, and blue dilution, *d*, in group II and with non agouti, *a*, and Danforth's short tail, *Sd*, in group V. (See Table II).

The evidence of linkage with *se* and *d* came entirely from coupling data, where it was largely due to a shortage of the triply-mutant phenotype; repulsion data failed to support

TABLE II. Independence tests with *Ra*

Linkage group	Marker gene, *M* or *m*	Mating type, phase*	Phenotypes of progeny *Ra M*	*Ra m*	+ *M*	+ *m*	Recombination percentage†
I	c^e, c^{ch}	BC	52	69	57	84	48.1 ± 3.1
"	*p*	BC	9	8	6	7	46.7 ± 9.1
II	*d*, *se*	BC	81	41	50	61	
"	" "	BR	15	10	17	16	41.9 ± 2.9
III	*s*	BC	63	60	67	45	54.0 ± 3.3
"	W^v	BC	81	93	81	89	50.6 ± 2.7
V	*a*	BC	32	8	16	35	
"	"	BR	17	35	40	8	25.7 ± 3.2
"	*Sd*	BR	14	28	25	15	35.4 ± 5.3
VI	*Ca*	BC	38	24	40	28	
"	"	BR	12	13	8	6	48.5 ± 3.8
"	*ht*	BC	8	4	7	1	55 (>32)
VII	*Re*	BC	9	7	8	10	
"	"	BR	28	21	15	29	56.7 ± 4.4
"	*wa-2*	BC	6	5	7	4	55 (>33)
VIII	*b*	BC	70	56	68	59	49.0 ± 3.1
IX	*T*	BC	1	1	1		
"	"	BR	60	35	47	37	54.4 ± 3.7
X	*v*	BC	3	2	1	4	30 (>7)
XI	Mi^{wh}	BR	16	9	9	5	53.8 ± 8.0
XII	*ru*	BC	2	1	8	10	43 (>22)
"	*je*	BC	2	1	9	8	50 (>28)
XIII	*fz*	BC	5		4	1	40 (>13)
"	*ln*	BC	5	6	6	4	57 (>35)
XIV	*f*	BC	1	2	12	3	78 (>47)
	Va	BR		1	2	2	60 (>6)
XX	♂	BC	21	25	26	18	
"	"	BR	59	80	70	82	50.4 ± 2.5

*B = backcross, C = coupling, R = repulsion. ♂ treated as a dominant.
†Where the total number of progeny classified exceeds 25 recombination percentage and its standard error are quoted; otherwise the recombination percentage and its lower (2½ percent) fiducial limit.

TABLE III. Three-point tests of *Ra* with *a^t* and *pa*

Genotype of parents	Phenotypes of progeny *Ra++*	*Ra+pa*	*+++*	*++pa*	*Ra a^t+*	*Ra a^tpa*	*+a^t+*	*+a^tpa*	Total
$+a^tpa/+a^tpa$♂ × *Ra+++/+a^tpa*♀	27	2	11	0	0	1	9	7	57
$+a^tpa/+a^tpa$♂ × *Ra a^tpa/+++*♀	2	0	6	1	1	3	0	1	14

TABLE IV. Three-point tests of *Ra* with *a* and *Sd*

Genotype of parents	Phenotypes of progeny *Ra+Sd*	*Ra++*	*++Sd*	*+++*	*Ra a Sd*	*Ra a+*	*+a Sd*	*+a+*	*Total*
+a+/+a+♂ × *Ra a+/++Sd*♀	1	0	1	0	3	2	3	1	11
Ra+Sd/+a+♂ × *+a+/+a+*♀	19	18	10	8	6	3	24	35	123

the suggestion of linkage. It is probably spurious.

The evidence of linkage with *a* was highly significant (25.7 ± 3.2 percent), with *Sd* less so (35.4 ± 5.3 percent). To establish the position of *Ra* in group V, three-point tests were made with pallid, *pa*, which lies between *a* and *Sd* (Borger[1]) and shows about 17 percent recombination with *a;* thus *Ra* would be expected to show close linkage with *pa* if the order were *a, pa, Ra,* but loose linkage if it were *Ra, a, pa.* The tests established without question that the order is *Ra, a, pa* (see Table III). Three-point tests with *Sd* indicated the order *Ra, a, Sd* (Table IV), confirming that *Ra* lies beyond *a* with respect to *pa* and *Sd; Ra* thus becomes an end marker of its group.

Joint estimates of the recombination percentages of *Ra* with *a, pa* and *Sd,* based on all available data, are given in Table V.

Non-Allelism of Ragged With Other Coat Texture Mutants

The location of *Ra* at one end of linkage group V establishes that it is not allelic with any of the known coat texture mutants. Only two of them belong to group V, *we* and *ro,* and both are located close to *pa* (Fisher,[6] for *we;* Falconer[4] for *ro*).

Discussion

Ragged promises to be an interesting and useful mutant from two viewpoints, those of formal and developmental genetics.

Its formal interest lies in the fact that it extends, by more than 20 units, a linkage group map that is already long. It is not surprising that group V should prove to be long, since Slizynski[7] has shown, through cytological study of a tagged translocation induced and analyzed by Snell,[8] that this group is carried in one of the longest autosomes. Its map is now of such length that it is no longer sufficient to use only the two end markers, *Ra* and *Sd,* when testing it for linkage with a new mutant.

The developmental interest of ragged lies chiefly in the opportunity which it affords for testing concepts about the development of the coat, and especially the hypothesis that each type of hair fiber arises from its characteristic type of follicle (Dry[3]) and that the various types of follicle appear in a time sequence (Falconer *et al.*[5]). Comparative studies of crinkled and normal mice by the latter authors showed that follicle initiation occurs in four main phases: (1) in embryos of 12½ to 13½ days, (2) in those of 14½ to 17½ days, (3) from 17½ days gestation to birth, and (4) in the first few days after birth. Follicles of the first phase give rise to sinus hairs, those of the third phase to awls and auchenes. Ragged resembles crinkled in having few zigzags, but differs in having plenty of guard hairs. It therefore constitutes ideal material for testing the remainder of the hypothesis, namely that the second phase of follicles gives rise to guard hairs and the fourth to zigzags.

TABLE V. Recombination of *Ra* with *a* or *a*^{t}, *pa* and *Sd*, from the data of Tables II, III and IV

Locus	Recombination with *Ra* and standard error (%)
a	24.2 ± 2.5
pa	39.4 ± 5.9
Sd	41.2 ± 5.3

Summary

Ragged, *Ra,* is a new semidominant coat-texture mutant in the house mouse. Heterozygotes have a sparse coat in which the proportion of zigzag fibers is low; in agouti mice the coat is abnormally dark, especially on the dorsum. Most homozygotes show a generalized oedema and die soon after birth; the few survivors are naked. *Ra* is located at one end of linkage group V; it shows 24 percent recombination with non agouti and 39 percent with pallid.

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MEASURING SHIPPING QUALITIES OF WATERMELONS

According to Snowden's Method

S. S. IVANOFF*

SNOWDEN'S METHOD

Figure 3

Watermelon rind strip from a non-shipping commercial variety is shown on the left. The rind is very brittle and breaks before it bends to make an arc of 60°. On the right is shown a rind strip from a well known shipping melon which remains unbroken after making an arc of 240°.

THE ability of a watermelon to ship well is a complex characteristic which depends upon several factors. Some of these seem related to the rind and its cell structure,[1,5,7] others to certain physical characteristics of the flesh.[1] Various mechanical means have been devised to measure rind breakage and "toughness" in watermelons.[1,2,6]

In breeding for disease resistance involving crosses between disease-susceptible shipping melons and resistant non-shipping melons, many fruits were found in later generations to possess a hard rind. These rinds remained rigid when pressure was applied to them by the thumb, the fingernail, or by any sharp instrument. It soon became evident, however, that not all melons with such hard rinds were good shipping melons because some of them broke easily by impact of the fist. They were hard but not "tough." It seemed that in selecting a melon for shipping, consideration of hardness of rind alone (as distinguished from softness), was not sufficient. There seemed to be something else in a rind, besides hardness, that made a melon suitable for shipping and hauling. This additional quality (the opposite to brittleness), may be called elasticity, flexibility, pliancy, or resiliency — a rubber-like quality giving the rind the ability to withstand physical stresses without breaking. Hardness plus flexibility, then, seemed to equal what we have been calling "toughness" in a watermelon rind.

History and Description of Snowden's Method

A very simple method for measuring this quality of resiliency in watermelon rinds has been employed by the writer for many years and has been found very useful. Until very recently,[4] however, the method had apparently remained unknown among watermelon breeders. The writer first learned of it many years ago from Mr. J. E. Snowden, (formerly Extension Marketing Specialist at State College, Mississippi, and at present, Manager, Factory Farming Company, Hattiesburg, Mississippi), and had mistakenly assumed that it was generally

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DEGREES OF TOUGHNESS AND PLIANCY
Figure 4

A model chart to measure geometrically degrees of toughness and pliancy in watermelon rinds. The most pliant rinds known are those of certain citrons which make a complete circle without breaking. (See text for explanation.)

known and applied. The publication of this method and the results of its application in breeding work by this writer has been suggested by other plant breeders who have recently given it a trial. It has proved very reliable in selecting tough-rind fruits in segregating populations.

The method consists of cutting out a thin strip or ribbon of rind between 1/16 and 1/8 of an inch thick and about three inches long. The strip is held at each end, with the epidermis facing the experimenter. With the strip in this position, the ends are slowly curved downward in an attempt to form a circle, until the strip breaks. The tougher the rind the more it bends without breaking.

After experimenting with this method for sometime, the writer made an attempt to standardize it so that it could measure elasticity in more or less exact geometric terms. The degree of arc formed when the breaking point is reached represents the relative degree of toughness. Very tough rinds, like the rinds of most citrons, may form a complete circle without breaking. On the other hand, rinds of non-shipping types of melons, whether hard or soft, break in a characteristic manner at the very start of bending, some even before making an arc of 60° (Figure 3). Other varieties of melons have rinds with breaking points falling somewhere between the two extremes, making arcs of approximately 120°, 180° and 240°, depending upon the variety.

The strip may be cut from any part of the melon fruit, either equatorially or meridianally. With notable uniformity in results, the test will show the resiliency of that particular section of the rind which is under test. However, fruits of most varieties possess rinds of more or less uniform quality throughout their circumference. Other varieties are known to have highly resilient rinds at the middle and stem-end sections of the fruits, but not at the blossom end. Snowden's method will bring out such differences, when they do exist.

Testing the Method With Melons of Known Rind Toughness

A number of well known commercial watermelon varieties and strains were tested by the standardized method in order to ascertain the degree of its reliability. A chart prepared to measure geometrically the resiliency of any rind is shown in Figure 4.

On the left is shown the complete circle made by the rind of citron. As is well known the citron has a very tough rind, it being both hard and rubbery. None of the rinds of commercial watermelons possesses the degree of toughness of citron. Next in degree of resiliency are a number of the best-known varieties of shipping melons, making an arc of 240°, i.e. Tom Watson, Congo, Wilt Resistant Dixie Queen, Florida Giant and Clara Lee. In this group also falls an F_1 hybrid made between Congo, a good shipping melon, and Miles, a melon of excellent epicurian qualities but with a soft and brittle rind. It has been previously reported that "toughness" of rind is inherited as a dominant character.[7]* In this case it appears that both qualities making for toughness, namely, hardness and elasticity, are dominant in their inheritance as determined by this test. In the F_2 generation it was found

*Dr. Porter actually determined the inheritance of explosiveness and non-explosiveness in watermelon fruits as a measure of toughness. In this writer's opinion explosiveness is the result of brittleness and non-explosiveness is determined by the high resiliency of the rind. Dr. Porter's conclusions, however, were correct.

TOUGHNESS IN WATERMELON
Figure 5

A watermelon is given a practical test for toughness. The melon will support a weight of over 200 lbs. without cracking. The rind of this melon, Dixie Queen Wilt Resistant (Mississippi strain), makes an arc of 240° without breaking. The rind is thin but both very hard and very pliant, which make for toughness. The flesh is very solid which adds significantly to the ability to withstand pressure and shipping.

---

that these two factors are inherited independently of each other although they may also be associated with each other. The inheritance of these characteristics cannot be expressed here on a factorial basis because of the small populations employed.

Next comes an intermediate 180°-arc group of watermelons which includes the varieties Hawkesbury, Blacklee, Leesburg, and a strain of Stone Mountain. Then follows the 120°-arc group including Cuban Queen and Strain Y of Stone Mountain, and finally, the 60°-arc group, composed of the non-shipping types, such as Halbert's Honey, Garrison, and Miles.

Tests have been made with both immature and mature melons, without significant differences in results as far as the writer has been able to determine. All these tests have been made with freshly harvested and freshly cut melons. It is common knowledge that when a melon is cut and then allowed to stand for prolonged time in the icebox or in the open, it loses moisture and the quality of its rind undergoes rapid changes. On the other hand, even though a melon may have been harvested some weeks before and hauled for some distance, the test may still be made with results showing the true shipping quality of the rind.

Possible Application of the Method to Other Problems

It is possible that hardness and elasticity may also be measurable characteristics making for toughness in rinds of other fruits and vegetables. It may be recalled that these two independent but often closely associated characteristics have long been recognized and measured in different species of trees as expressing the commonly known "toughness" of woods.[3] It might even be profitable to approach the problem of lodging in corn, oats, wheat, and in other cereals and grasses from the point of view of hardness, brittleness, as well as flexibility, of tissue. It may be worthwhile to investigate the problem of what physical-chemical properties of cells, or what colloidal contents of tissues, might determine flexibility.

Summary

A simple method for measuring shipping qualities in watermelon fruits is described. It consists in bending a thin strip of rind into a circle until it breaks. The degree of arc formed by the strip when the breaking point is reached represents the relative shipping ability of the melon.

The employment of this method named by the writer (Snowden's method), has led to a better understanding of the nature of the so-called "tough" rinds in shipping melons. "Toughness" seems to consist of two equally important and independently inherited components: hardness and resiliency. The two characteristics were often found associated with one another in the F_2 generation, as they are found associated in commercial varieties of the shipping type. Both characteristics were inherited in a dominant manner.

An anology is pointed out between the elements of "toughness" in watermelon rinds and those in the wood of trees. In both cases the two elements, hardness and elasticity, are involved. It is suggested that the problem of shipping qualities in other fruits, as well as the problem of lodging in certain field crops, be re-examined from the point of view of both hardness and elasticity of tissues.

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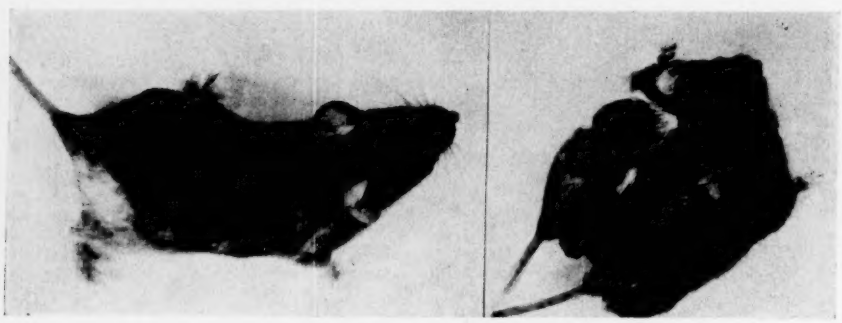
The Tortoise Shell House Mouse

Margaret M. Dickie*



TORTOISE SHELL FEMALES
Figure 6

The tortoise shell mouse is characterized by its vari-colored coat which consists of black, brown, yellow, grey and almost white. The character, affecting only females, appears to be due to a dominant sex-linked gene.

SEVERAL variegation patterns have been described in mice, among them the varitint waddler (*Va*),[1] an autosomal mutation, and tabby (*Ta*)[2], brindled (*Br*) and mottled (*Mo*)[3], all sex-linked mutations. The new character to be described resembles a color pattern in cats as does the tabby mouse. This new character also appears to be similar to brindled and mottled and breeds somewhat like mottled.

In the summer of 1952 an odd colored animal was observed in a litter of the obese stock. This animal, a female, was vari-colored: black, brown, yellowish, grey and almost white. Because of its similiarity to the tortoise shell cat, though with less yellow, this character was named tortoise shell.

When this female tortoise shell mouse was mated with a normal colored (black) sib, some female tortoise shell animals occurred among the F_1 generation. These females as well as normal colored ones have been mated with their brothers. The tortoise shell females produce both tortoise shell and normal colored female offspring, but the normal colored females produce *only* normal colored offspring. Further, no tortoise males have yet been observed, and combining all data, there is a deficiency of male offspring (Table I). This suggests that this may be a dominant sex-linked gene, lethal when not balanced by its normal allele.

Only one outcross has been made and that

(*Continued on page* 190)

*Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine. This work was supported by grants to the Jackson Memorial Laboratory from the Anna Fuller Fund, the Jane Coffins Childs Memorial Fund for Medical Research and the National Cancer Institute of the National Institutes of Health, Public Health Service.

COLCHIPLOIDY IN GRAPES

HAIG DERMEN*

LARGE-SIZED table grapes are generally considered more attractive and bring higher prices than smaller ones. Polyploid mutants of varieties with berries twice the normal size have been found. Thus, the development of polyploids by colchicine treatment would seem to be a promising way of improving grapes. Polyploidizing certain varieties may give the immediate results desired without adverse changes in their fertility and ordinary cultural requirements. However, for general breeding purposes, many selected varieties, known for fruit quality, resistance to disease, vigor of growth and other desirable qualities, should be polyploidized so that they will be available as breeding material. Breeding and selection may then be carried on at a tetraploid level and increased berry size in the progeny will be assured. The breeder could then concentrate on the development of other desirable qualities just as when breeding is at the diploid level.

The present method of polyploidizing may also be used to solve a challenging problem in grape breeding. Hybrids obtained when plants of two species are crossed are often completely or partially sterile. Some of these hybrids are made fertile when they are polyploidized; or fertile hybrids are obtained when one or both parents are polyploidized and then crosses are made between the polyploid forms. Crosses between varieties of the European grape, *Vitis vinifera*, and of *V. labrusca* with the muscadine grape, *V. rotundifolia*, have always resulted in highly or completely sterile hybrids. Therefore, in the present breeding program attempts are also being made to produce fertile hybrid grapes by crossing the tetraploid forms of these species.

Unusual success has been achieved in inducing polyploidy in grapes by the use of colchicine solution. The methods used in the diagnosis and isolation of tetraploid branches, described herein, were in part responsible for the successful outcome. Grapes grow at a rapid rate under greenhouse conditions and whenever branches are cut back, new growth from lateral buds can be easily obtained—a very favorable situation for forcing outgrowth of polyploid branches. A number of features that showed the presence of polyploid branches on the treated plants were helpful in the diagnosis of induced ploidy. Because of the success obtained in these experiments and the possibility that the experience gained from this work may be of benefit in conducting similar experiments with other plants, a number of the most essential features used for identifying polyploid grapes are illustrated in the paper.

Colchicine experiments with grapes have extended over two years. In 1952 the work was with American bunch grape varieties adapted to the northeastern United States. In 1953 certain muscadine grapes adapted to the southern states and one *V. vinifera* variety were used. Plans for 1954 are to work with varieties of *Vitis vinifera*, some additional muscadine grapes and some sterile hybrids between bunch grapes and muscadines. Crosses will be attempted between the tetraploids of the bunch and muscadine grapes to determine whether hybrids of the tetraploids of the two types will be fertile, since hybrids between the diploids have been sterile.

Material and Technique

Colchicine treatment was begun May 19, 1952, on 10 varieties and selections of American bunch grapes adapted to northeastern regions of the United States. Subsequent results indicate that it would have been better had these plants been treated two months earlier. Material for this work was selected and provided by Donald H. Scott of this Station. Varieties and selections used were Champanel, Champion, Dakota, Franklin, Loretto, Seneca

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THE METHOD OF COLCHICINE TREATMENT

Figure 7

A—shows a grape plant (Fredonia variety) prepared for treatment. The tip of the pipette can be seen at the upper left and the bottle of colchicine solution at the right. (×½). *B*—Upper portion of the plant in *A* before preparation for treatment. The very tip of the shoot and the buds below, where the tag is tied, were cut off (compare with *A*). Three buds were left for treatment. (Approximately × 1.) *C*—The plant in *A* treated five times and photographed 12 days after the fifth treatment. Upper bud with its basal stem part has abscised and the remaining two buds have grown. A secondary bud at the base of the top bud has also grown. (× ¼.) *Photographed by Otis H. Greeson.*

(a large-fruited and vigorously growing strain and a normal or smaller-fruited one with less vigor of growth), Vergennes and selections Nos. 519-6 and 520-2. Ten plants of each were treated.

Treatment of muscadine grapes was begun March 10, 1953. On that date young shoots on a few rooted cuttings were at the proper stage of development for treatment, and others were ready on later dates, extending into late April. The following six varieties and six selections of muscadines were furnished by N. H. Loomis of the U. S. Horticultural Field Station, Meridian, Mississippi: Creek, Dulcet, Scuppernong, Thomas, Topsail, Yuga, Sels. 7 and 10, Nos. 6-4A, 14-5A, 47-9B and 51-9B. An additional five muscadine selections were furnished by C. F. Williams of the North Carolina Agricultural Experiment Station: Nos. 5-106, 11-178, 17-123, 17-197 and 20-30. The number of plants of each variety and selection for treatment varied from 10 to three. In addition three plants of Blackrose, a *V. vinifera* variety, and one plant of the American bunch grape variety Fredonia were treated June 12. Plants of the last two varieties were treated at a late date mainly to provide illustrative material (Figures 7 and 8).

Originally it was thought that 10 plants of each variety should be treated in order to ob-

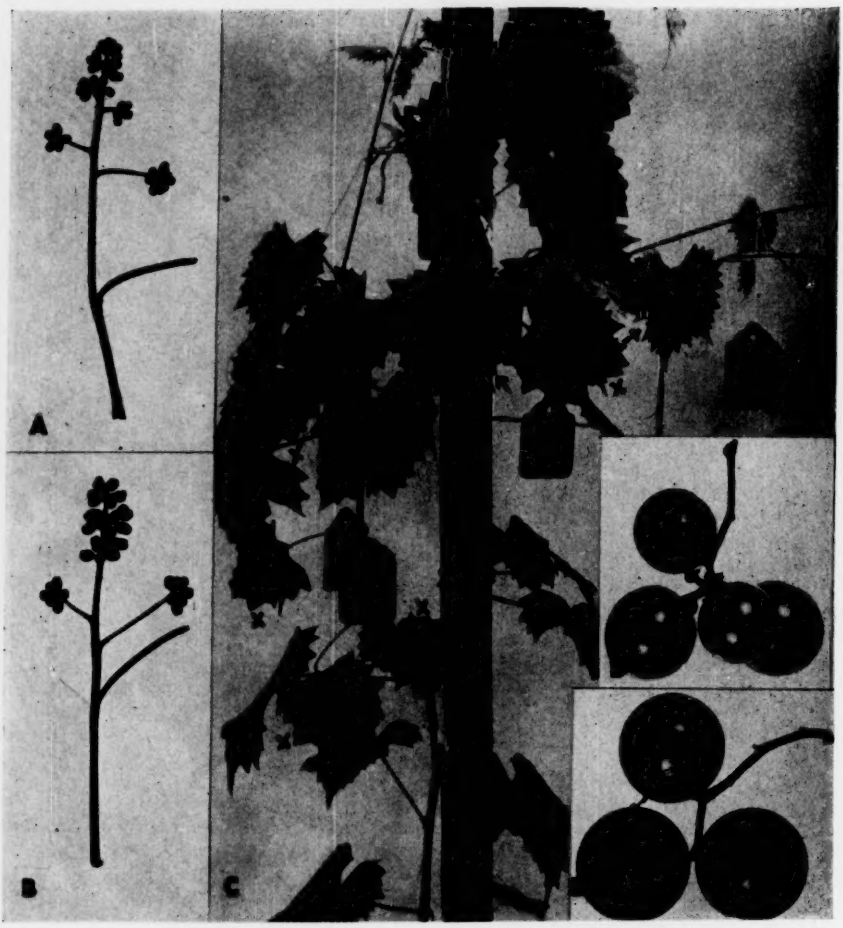
TREATED AND UNTREATED PLANTS
Figure 8

These two plants (Blackrose variety) were prepared for treatment by cutting the tips off and digging out all buds except three at the upper portion. The plant on the left was not treated and the plant on the right was treated every other day for a total of five times. Both plants were photographed 12 days after the last treatment of the treated plant. Lateral shoot growth can be seen from three buds on the untreated plant. Shoot growth from treated buds was retarded. *Photographed by Otis H. Greeson.*

tain at least one plant with internal polyploid tissues. The results obtained indicate that five plants of each would have been sufficient for a high probability of success of obtaining branches with all tissues tetraploid.

The solution used was 0.5 percent colchicine in 10 percent glycerine in water. Glycerine is used to provide a non-volatile medium which will keep the treated area moist and hold colchicine in place to be absorbed gradually. Higher percentages of glycerine are injurious to many plants. To each 10 ml. of the solution were also added two drops of 10 percent Santomerse (a spreading reagent, produced by the Monsanto Chemical Co., St. Louis, Missouri). A small crystal of thymol, 1 to 2 mm,[3] was put in the bottle containing 20 ml. of solution to prevent growth of contaminants. Incidentally, the solution used in 1953 was a year old and was found to be still potent—a matter of practical consideration. Because of the manner in which the colchicine solution was applied in these experiments, its cost was only a few cents.

In 1952 unrooted cuttings, each with three buds, were placed in 8-inch pots with two

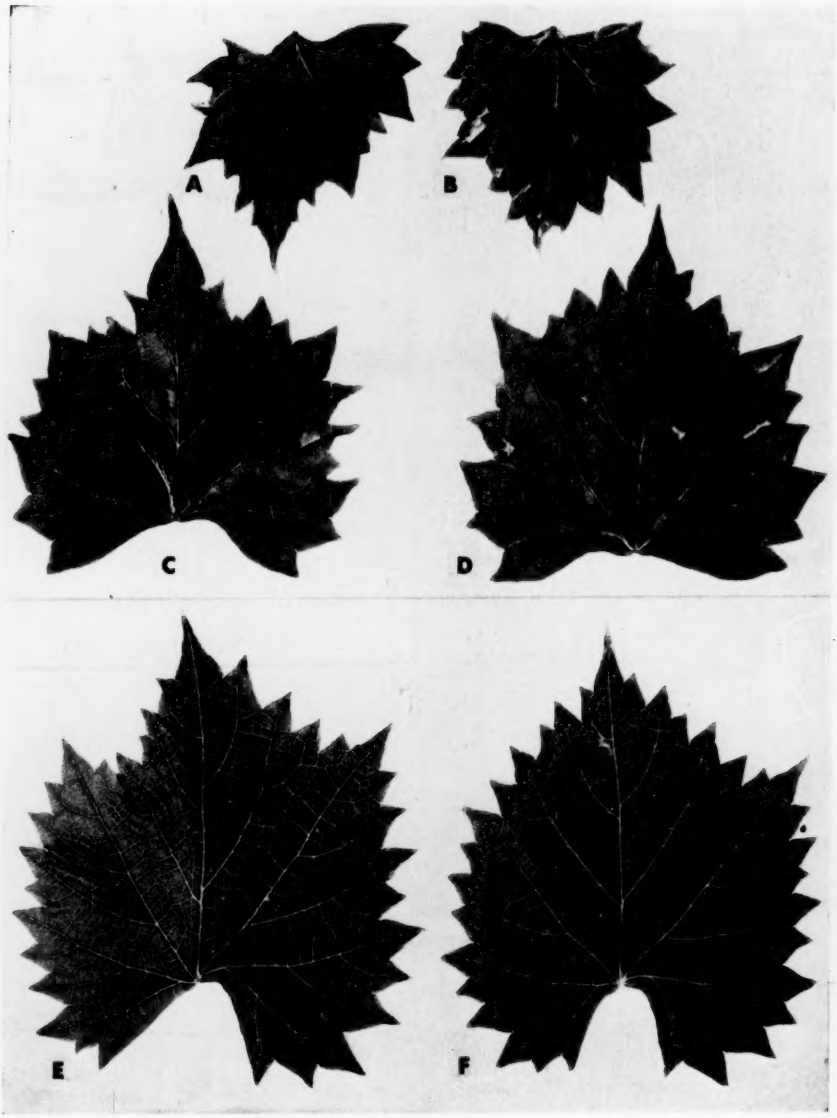


DIPLOID AND TETRAPLOID MUSCADINE GRAPE

Figure 9

A and *B* shows a diploid and a tetraploid muscadine (No. 6-4A) grape flower cluster, respectively. (× 1.) *C*—shows a muscadine grape plant (No. 11-178) treated when at the same stage of growth as those plants shown in Figures 7 and 8. It was treated three times and photographed 21 days after the last treatment. Tags are tied to the stems below leaves showing sectorial polyploidy. See text for explanation of arrows and cross marks. *D* and *E*—A diploid and tetraploid muscadine (No. 6-4A) fruit cluster, respectively. (× 1.) *C—photographed by Otis H. Greeson; A, B, D* and *E photographed by Robert L. Taylor, Sr.*

buds above ground. The best growing shoot was retained and the others were cut off. Treatment was initiated after the shoot reached a length of about six inches. Figure 7*A* shows a grape plant (Fredonia variety) after it was prepared for treatment. In preparing the shoot for treatment, the very tip of the shoot and all the buds below the tag were cut off, leaving only three upper buds to be treated. In Figure 7*B* is shown the upper portion of the same plant (normal size) before the plant was prepared for treatment. This figure shows the very top of the plant and the two buds below the tag before they were cut off. Other buds



MOSAIC PATTERNED LEAVES

Figure 10

A-D—Mosaic patterned dark and light green leaves from the muscadine grape shown in Figure 9C; they appear somewhat distorted because of the excess colchicine effect. Dark areas are presumably polyploid and light areas diploid. *E* and *F*—Sectorially polyploid leaves; the light areas are diploid and the dark areas tetraploid. *Photographed by Francis J. Krakosky.*

at lower points are not shown. The forked bodies along the branch are the tendrils. Usually three axillary buds were left in the upper portion of the plant, but in a few cases four or five buds were left and treated.

The colchicine solution was applied with a pipette. The regular procedure was to wet the buds with a small drop or so of the solution once every two days for a total of three times. In a few cases application was made four or five times. Just enough solution was applied to each bud to fill the space between the base of the petiole and the stem and to barely cover the bud. An effort was made to prevent the solution from running off, but occasionally the liquid flowed down the stem, in which case the treatment was repeated at shorter intervals. In some instances, because of the nature of the stem surface the solution will not stay in place, a small amount of paper or cotton may be placed over the buds to hold the liquid so that it can gradually be absorbed. Superficial wetting of buds cannot possibly induce polyploidy in the growing points of buds well covered with bud scales and young leaves.

The treated bunch grapes were kept in the greenhouse until late October, 1952. Heavy string was tied below the first sectorially affected leaves on those branches which bore such leaves. Branches thus tagged were cut back to sectorially affected upper leaves. On April 30, 1953, these were planted in the vineyard. During storage shoots 1 to 2 inches long had grown from almost all of the treated buds. As these young shoots were almost colorless and very brittle planting was handled with extreme care. Fortunately the few days during and following planting were cloudy, and the etiolated branches had sufficient time to become normal green.

The treated muscadine grapes were grown in the greenhouse until the end of September, 1953. From then until the middle of November they were kept at lower temperatures for partial hardening. Then they were taken to the Agricultural Experiment Station at Raleigh, N. C., for permanent care and culture.

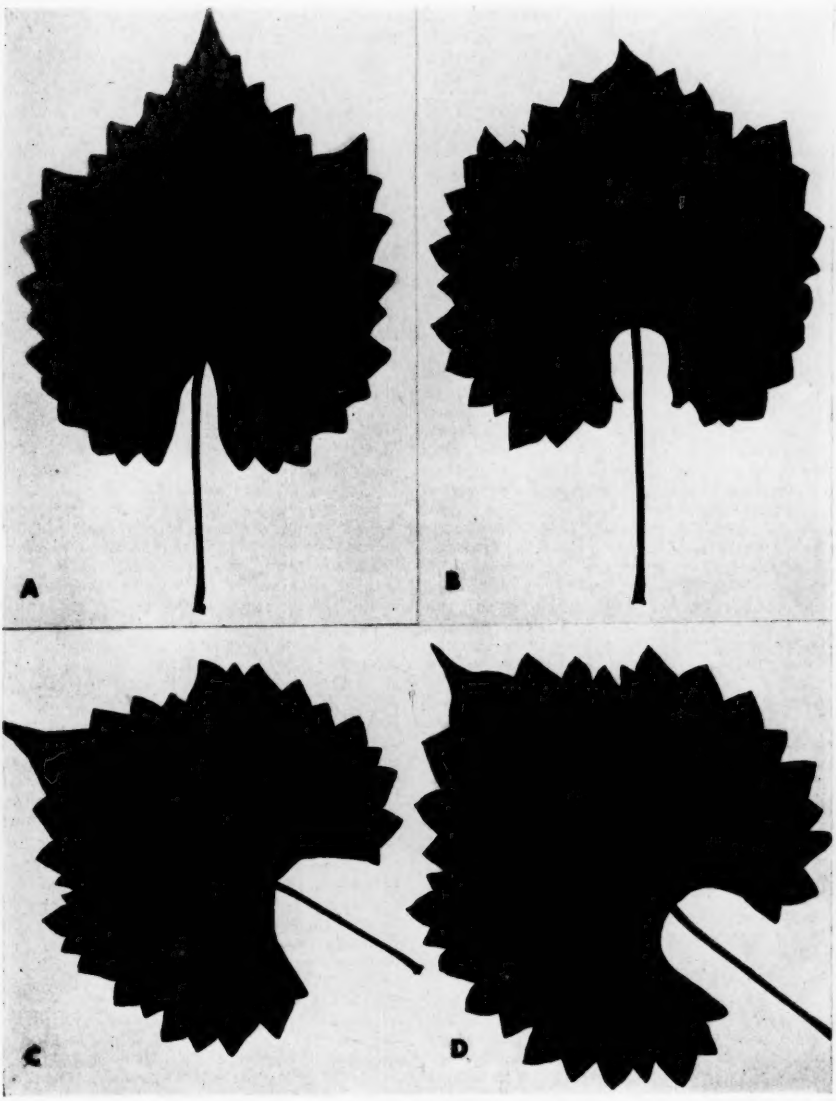
The diploid nature of the plants used in the present experiment was known in some cases by their breeding behavior and in others by comparison of pollen grain size with those of the parents from which they were derived. After treatment the tetraploidy of the epidermis was determined by stomate measurements and the tetraploidy of the sexual tissue by the pollen grain size. Such measurements have proven many times to be directly related to the degree of ploidy in numerous species of plants, with very rare exceptions. In this work the determination of ploidy was based entirely on such measurements, and these proved adequate.

Diagnosis of Induced Polyploidy

When the colchicine solution is applied to a bud or a shoot tip, certain physiological, cytological, histological, and morphological changes may occur in the treated areas. As a result of such changes certain features appear in the new growth from the treated material. Some of these are apparent to the naked eye and others are determined microscopically, as described below.

1. *Retardation of shoot emergence.* The first noticeable effect of the treatment was a retardation of shoot growth from the treated buds as compared with growth from buds on an untreated plant. In Figure 8 are shown two young vines of the variety Blackrose prepared in the same manner as the plant shown in Figure 7*A*. The upper three buds of the shoot in Figure 8 (left) were not treated, whereas the upper three buds of the shoot in Figure 8 (right) were treated. The first application of colchicine was made on June 12, and four subsequent applications were made on June 13, 15, 17, and 19. Both vines were photographed July 1, twelve days after the last colchicine application. The plant shown in Figure 7*A* was treated at the same time as the plant shown in Figure 8. Some growth on the plant shown in Figure 7*A* had occurred during the twelve days (shown in Figure 7*C*). Apparently because of the severity of the treatment—five applications instead of the usual three—the upper bud on this plant with the internodal stem below it abscised; thus growth had taken place from only two buds. Later the plant in Figure 8 grew freely and that in Figure 7*C* grew less freely. Subsequently polyploid branches were found on the plant shown in Figure 8, in the late summer of 1953, and on the plant shown in Figure 7 in the early spring of 1954. Where the applications of colchicine were limited to three, growth was less retarded during a similar time interval than in the plants shown in Figures 7*C* and 8.

2. *Mosaic pattern of ploidy in leaves.* On a vigorously growing untreated shoot, the first few leaves remain smaller than leaves which develop at a later date, but are otherwise quite normal in form; whereas the corresponding leaves developed on the shoots growing from the treated buds may show certain abnormalities. Thus leaves on affected shoots show whether the colchicine solution has penetrated into the buds and the treatment has been effective. Central growing points of shoot tips are enclosed by a dozen or more scales and very young and primordial leaves. Besides the central growing point of each bud there are, at the axils of bud scales and leaves, other potential growing points which can develop into shoots. The aim is to have the colchicine solution reach all the growing points. The solution must come in contact with the bud scales and young leaves in which cell division is active before it can reach the growing points; consequently bud leaves become affected. As shoots develop from treated buds, and as young and primordial leaves grow to maturity, certain striking features become notic-



DIPLOID AND TETRAPLOID LEAVES

Figure 11

Two pairs of leaves from two different muscadine selections. *A* and *C* are normal diploid leaves; *B* and *D* are tetraploid leaves. The U-shaped base at the point of blade and petiole attachment in *B* and *D* characterizes tetraploid leaves and was used as a method of identifying tetraploid branches. *Photographed by Otis H. Greeson.*

able: 1) The first few basal leaves that appear are distorted and 2) these leaves have a mosaic pattern of different shades of green; some patches are dark green and other patches are light green. Examples of such leaves are shown in Figure 10*A-D*; they were taken from the plant shown in Figure 9*C*. The treatment of this plant was initiated March 17, 1953, and was photographed on June 12. On this plant five buds were treated. Following the treatment the uppermost bud and the internodal stem below it died. Shoots grew from each of the next three buds, but no shoot developed from the lowest treated bud. The two upper shoots have at their bases a number of mosaic appearing leaves as shown in Figure 10 (cross marked in Figure 9*C*). The lowest branch did not show the mosaic pattern, was presumed to be lacking in polyploidy and was therefore cut off.

3. *Sectorially tetraploid leaves.* It appears that in a great majority of cases shoots growing from the treated buds are not wholly polyploid; they are only sectorially polyploid. This sectorial effect may be recognized by contrast in the appearance of the leaves. Two examples of sectorially polyploid leaves are shown in Figure 10*E* and *F* and Figure 9*C* (marked by arrows). The darker portions of the leaves indicate that the leaves and shoot along that side are tetraploid and the remaining portions of both leaves and shoot are diploid. Sectorial leaves were found along the branches, past the mosaic leaves, and were tagged. On the tag was noted which side of the leaf (whether the right or left) and what portion was tetraploid; i.e. L ¾ + (left side, a little over three-fourths tetraploid). In 1952 the branches of the eastern bunch grapes bearing sectorial leaves were thus tagged; and before the plants were placed in winter storage, these branches were cut back to sectorial leaves located toward the ends of the branches. At all times branches that appeared to be normal were pruned off. Thus, it was hoped that new branches would develop from the axillary buds of the sectorial leaves when the plant was set in the vineyard in the spring of 1953 and by chance from the buds at axils of leaves that may have been totally tetraploid.

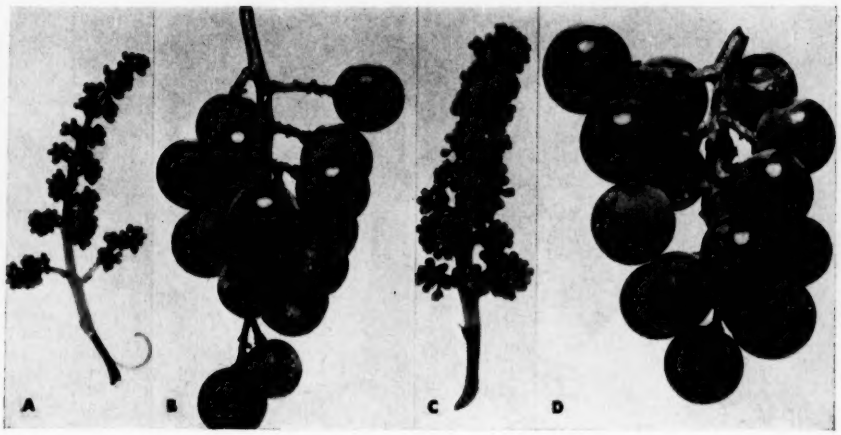
4. *U-shape character of leaves at blade and petiole attachment point.* During the polyploidy work with muscadine grapes in 1953 a very useful feature was discovered which helped to identify branches that were totally tetraploid. This consisted of a morphological change in the shape of the curvature on the tetraploid leaves at the blade and petiole attachment point. In Figure 11 are shown two pairs of leaves (*A* and *B*, *C* and *D*) from two separate grape selections. Normally varieties or selections differ somewhat in the shape of their leaf bases. In some, as in Figure 11*A*, a narrow angle is formed at the blade and petiole attachment point and in others, as in Figure 11*C*, the angle is wide and the base of the blade is more or less straight or is sharply bent at some points. In some other plants the base of the leaf is much wider and the lower leaf margin is almost straight. In contrast with normal leaves, the base at the point of attachment to the petiole is curved in the shape of the letter U (Figure 11*B* and *D*) in leaves of totally tetraploid branches. A partial change in curvature was also found in the sectorially tetraploid leaves. In such leaves (Figure 10*E*, especially) the edge of the blade at the petiole attachment point on the tetraploid side (darker portion, at the right) was more curved than on the opposite normal side.

5. *Sizes of flower clusters, individual flowers, pollen grains and leaf stomates.* In 1952 when work was conducted on bunch grape varieties grown in the greenhouse, tetraploid branches were identified by sectorial leaves alone and only in one or two instances were flower clusters borne. In 1953 most of the plants of bunch grapes in the vineyard produced flower clusters. Pollen shedding took place between May 25 and June 4. Some flower clusters on branches that were marked for polyploidy on a sectorial leaf basis were noticeably larger than those on check plants or on many other branches that were not marked, because of the absence of sectorial leaves. Pollen grains from the larger clusters measured 30 to 32μ and those from slender or normal appearing clusters measured 24 to 26μ, signifying that large clusters surely represented the tetraploid branches. In Figure 12*A* and *C* are shown two flower clusters; the one in Figure 12*A* is diploid and the one in Figure 12*C* tetraploid. It appears that there is no appreciable difference in number of flowers between the two clusters; and that the larger appearance of the tetraploid cluster is mainly due to the large size of individual flowers in the cluster but is also due in part to the bulkier stem structure of the tetraploid cluster.

Most colchicine treated plants of the muscadine grape produced flowers on branches which developed after treatment. The relation of flower size to ploidy previously described was also clearly seen in these plants. Size of pollen grains of both the diploid and tetraploid were of the same magnitude as in bunch grapes. Two flower clusters of a muscadine grape selection, No. 6-4A, are shown in Figure 9*A* and *B*. The cluster in Figure 9*A* is diploid and that in Figure 9*B* is tetraploid.

It may be of interest to note here that after the ploidy of branches was confirmed on the basis of flower and pollen grain sizes the U-character of the leaves was discovered as an important feature of the tetraploid branches. After this discovery the isolation of tetraploid branches was mainly based on the U-character.

The size of stomates was studied only in muscadine grapes. A hand lens magnifying 16× was used for examining the stomates. Stomates of the normal grape leaves were found to be of uniform size in contrast to the



FLOWER AND FRUIT CLUSTERS
Figure 12

A and *C*—A diploid and a tetraploid flower cluster, respectively, in a bunch grape selection No. 520-2. (× 3/5.) *B* and *D*—A diploid and tetraploid fruit cluster, respectively, on a first year growth of grape No. 520-2. (× 3/5.) *Photographed by Robert L. Taylor, Sr.*

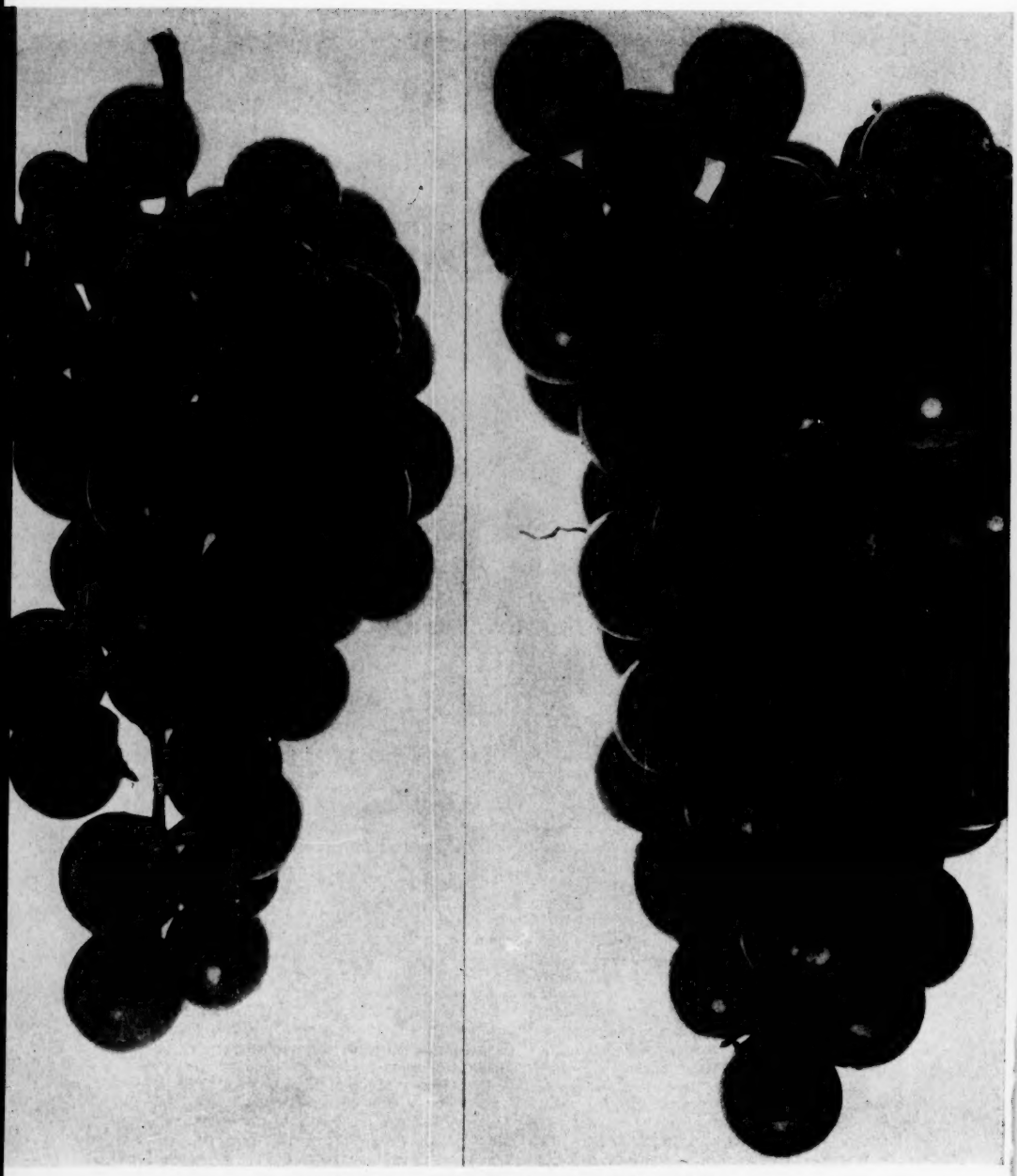
irregular size of stomates in the apple.[5] Branches with definitely larger stomates than normal were noted in six varieties and selections in which plants were also tetraploid internally. Plants with large stomates, large pollen grains and the U-shaped leaf bases were marked 4-4 and those with a normal epidermis but were tetraploid internally were marked 2-4 (the numeral 2 designating diploidy and 4 tetraploidy).

6. *Fruit size and fertility.* Fruit matured on some of the field-planted bunch grapes and on some of the greenhouse grown muscadines. There was a definite increase in fruit size due to internal tetraploidy. The two bunches of grapes shown in Figure 12 were from selection No. 520-2. The bunch in Figure 12*B* was from a diploid branch and that in Figure 12*D* from an internally tetraploid branch; ploidy of the epidermis was not determined. Both bunches have fewer fruits than flowers, and the bunches appear to be not much longer than the flower clusters. This particular selection was known to have normally loose bunches, due perhaps to a low degree of fertility. It will be of great interest to know whether fertility of this selection and of some others will be improved when tetraploid plants become more mature. In Figure 14 are shown a diploid and a tetraploid bunch of the variety Dakota. In this case the number of fruits per bunch did not vary greatly from the number of flowers per cluster.

Muscadine grapes are known for the small number of berries per bunch. The bunch in Figure 9*D* shows normal sized berries of a diploid bunch of selection No. 6-4A and the bunch in Figure 9*E* the much larger fruit of a tetraploid bunch on the same plant.

7. *Tetraploid sports. Vitis vinifera*, represented by so-called California or European varieties, *V. labrusca* and other species of the northeastern United States bunch grapes have $2n = 38$ chromosomes.[9,11] *V. rotoundifolia*, the southern muscadine grape varieties have $2n = 40$ chromosomes.[11] Nebel determined the chromosome number of large-fruited sports of Muscat of Alexandria and Sultanina varieties to be $2n = 76$ chromosomes. The present writer in 1938 determined the tetraploid chromosome number of Eaton variety from a root tip study. Large pollen grain size in this variety led to this discovery.[1] Olmo[10] gave a brief account of the tetraploid sports of other varieties. Einset and Lamb[7] determined cytohistologically the periclinal chimeral nature of many large berried sports, including Eaton. They remarked: "Most of these have been of the Concord and Fredonia varieties. Chromosome counts in root tips have given the tetraploid number but examination of buds has shown in each instance that the epidermal layer has been diploid. In some of the sports diploid cells have also been found in deeper tissues." They state further that ". . . perhaps all the tetraploid grapes are diploid-tetraploid periclinal chimeras rather than wholly tetraploid forms."

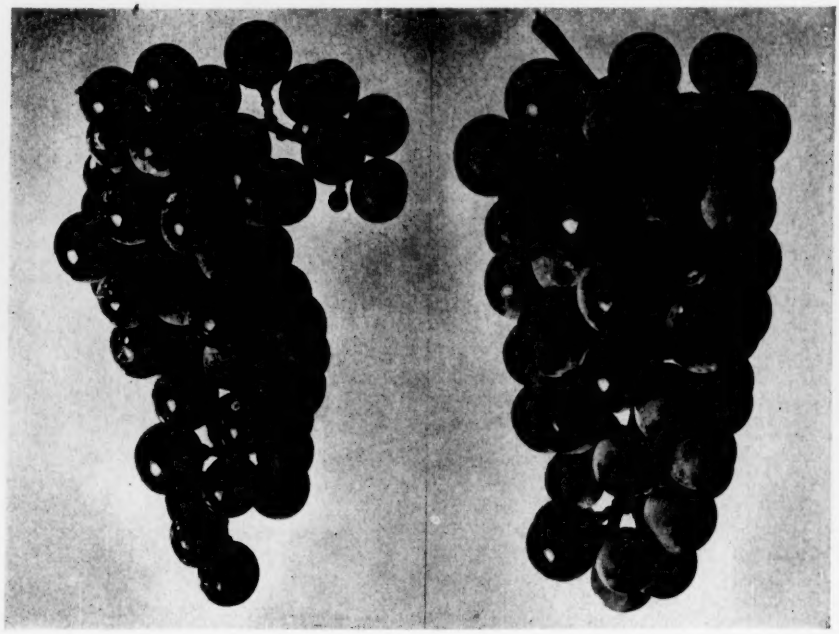
In 1948 the cytohistology of a large-fruited sport of Portland and of Wallis Giant and Starks Early Giant varieties was determined. The epidermis in all three was diploid and the internal tissues were tetraploid. Hence, all three must have originated as sports of diploid



DIPLOID AND TETRAPLOID BUNCH GRAPES

Figure 13

A diploid (left) and a tetraploid (right) fruit cluster, from a commercial variety, Portland. The tetraploid is a natural sport. The fruit cluster of the tetraploid is definitely larger than that of the diploid. (Approximately × 1.) *Photographed by Otis H. Greeson.*



DIPLOID AND TETRAPLOID DAKOTA VARIETY

Figure 14

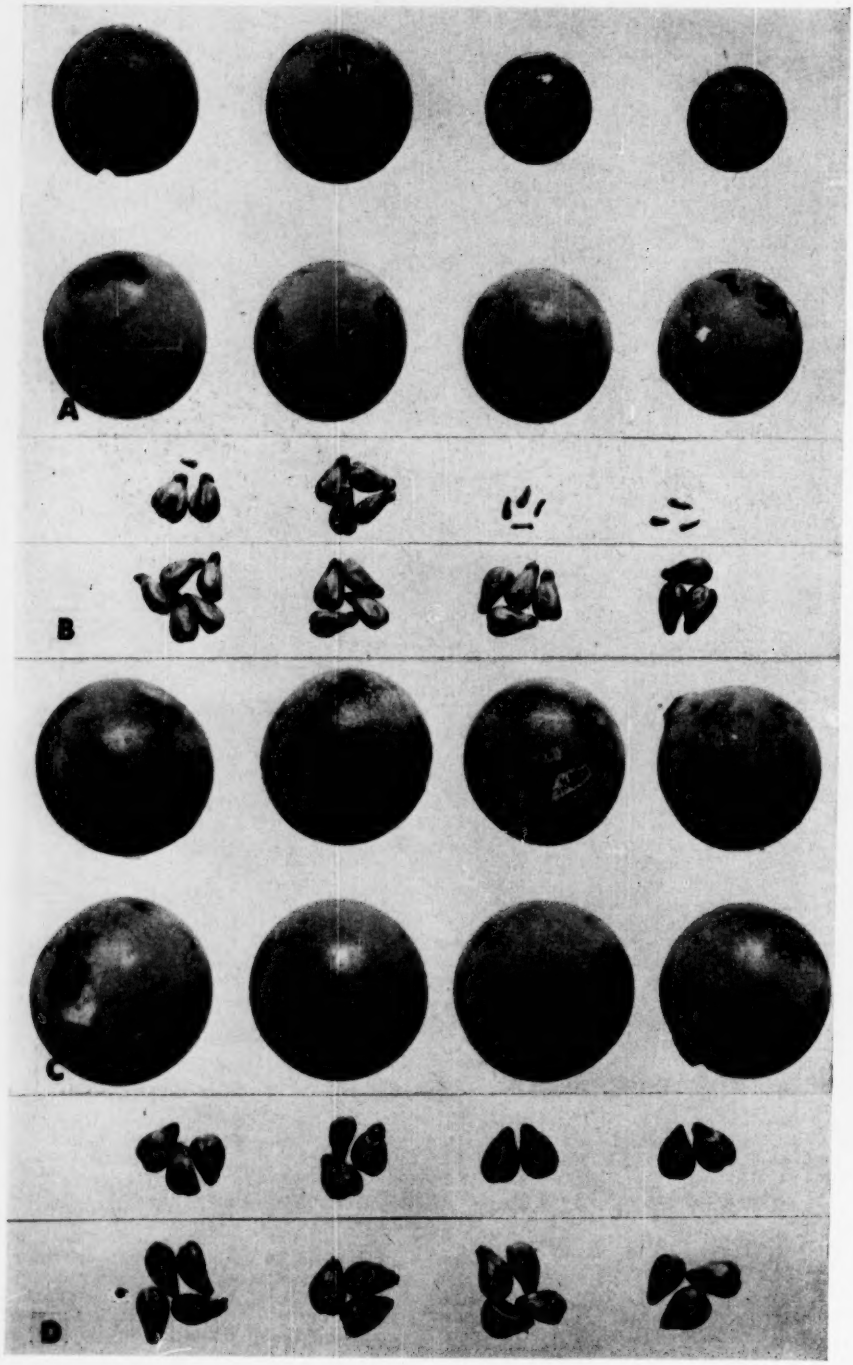
A diploid (left) and a tetraploid (right) fruit cluster, from a commercial variety, Dakota. These clusters from one-year-old plants compared very favorably in size with those from older plants. (× 7/10.) *Photographed by Robert L. Taylor, Sr.*

varieties. All three have produced good fruit clusters. A diploid and a tetraploid bunch of Portland are shown in Figure 13. Normally the bunches of the diploid Portland were loosely filled and in addition some of the berries were small. On the other hand, the bunches of the tetraploid were fuller and the fruits were all of the large size. In Figure 15*A* are shown examples of diploid fruits and in Figure 15*C* examples of tetraploid fruits. Each group of fruit was picked from a single bunch and a single area in each bunch shown in Figure 13. Seeds are shown directly below the fruits in Figure 15*A* and *C*. These illustrations show that in the Portland variety tetraploidy has increased the size of the fruit and the seeds are broader than those of the diploid. The seeds of the two small fruits of the diploid have aborted. Lack of aborted seeds in the tetraploid fruits sampled may indicate improvement of fertility by polyploidy in this particular variety. Good fertility was indicated in some colchicine-induced tetraploid forms, but others may not be as fertile as the diploid.

Olmo[10] carried on extensive breeding experiments with tetraploids and has made many valuable observations in this respect. For example, he has noted that ". . . it has been possible by breeding to obtain tetraploid varieties differing markedly in vigor, fruitfulness and fruit quality." He found that some undesirable characteristics associated with tetraploidy may be eliminated by crossing and selection, and still maintain the desired larger berry size.

Discussion

It has been shown in numerous instances in other plants that size of fruit is increased by chromosome doubling. The grape is a plant in which larger berry size is highly desirable. The results reported here show that tetraploidy in grapes can be induced with ease if proper methods are employed. In 1952, 10 varieties and selections of northeastern bunch grapes were treated and it appears from the characters described above that tetraploid plants of all 10 were produced. Tetraploidy was determined in all except two varieties of bunch grapes by pollen grain size. Tetraploidy in two varieties, Champanel and Seneca, was determined by leaf characters.


A
B
C
D

In 1953, 17 varieties and selections of muscadine grapes were treated. Tetraploidy was induced in 16; the failure occurred in one for which there were only three plants available for treatment. Three plants of the variety Blackrose (*V. vinifera*) were treated and leaf characters of a number of branches indicated that they were tetraploid.

In 1952 the isolation of tetraploid branches in the bunch grapes was based chiefly on sectorial leaf character, which is not too easy to detect. In 1953 tetraploidy was based on size of flower clusters and pollen grains. In the muscadines after many branches were tagged for polyploidy on the basis of sectorial leaf and of size of flower cluster and pollen grains, the U-shape character in the leaf bases was noted and was found by examining flowers on tetraploid canes to be perfectly correlated with the other criteria of tetraploidy.

At the time the U-character in muscadines was discovered there were many plants which were still in vigorous growing condition. After that, plants were closely examined, tetraploid branches recognized by U-character were tagged, normal branches were cut off and new branches were allowed to grow at the axils of sectorially tetraploid and mosaic leaves. As the new branches emerged their ploidy was quite easily recognized on the basis of whether their leaves had a U-shape or an angular base. The normal-leaf branches were cut off as soon as their nature was determined. It was remarkable that these grapes could be cut back so many times and still generate new growth. It should be pointed out, therefore, that the success in obtaining polyploidy reported here was in great measure due to the facility of regeneration in these plants. It is possible that similar results might be obtained with other species of plants, especially woody plants, if they could be made to regenerate shoots as grapes do when unaffected parts of the plants are cut back. If this cannot be done in one season with certain species of plants, then the process of cutting back to regenerate new growth will have to be extended into the second or even third season of growth. Failure in obtaining polyploids in some colchicine treated plants may be associated with lack of regeneration, discussed above. Also, great effort should be made to detect the presence on the treated plant of any abnormalities, such as structural deformities and morphological differences in the leaves, which may be associated with polyploidy. Such abnormalities would be very helpful in the diagnosis and isolation of polyploid branches on the treated plant.

Another remarkable feature of the muscadine grape is that many branches grow from treated buds and produce flower clusters and ripe fruits during one growing season. After branches were identified as being internally tetraploid, stomates of the leaves were then examined. With the aid of all characteristics used in the diagnosis of ploidy, branches were labeled accurately as being a) 4-4, both epidermally and internally tetraploid, b) 2-4, epidermally diploid and internally tetraploid, and c) 4-2, epidermally tetraploid and internally diploid. No special effort was made to determine the presence of the last type branches; the discovery of these was incidental.

In breeding on a tetraploid level both 4-4 and 2-4 types may be used. But the breeder should be cautioned about the use of 2-4 type plants. In some cases internal tissue may develop from a diploid external layer of cells, thus changing the reproductive tissue from tetraploid into a diploid condition. Olmo[10] reported the occurrence of this in the grape. The same phenomenon appears to have occurred in diploid-tetraploid material studied by Einset and Lamb,[7] but they failed to point that out. This phenomenon seems to occur rather regularly in some monocots.[8, 12] The basis of this kind of change has been explained elsewhere.[3, 6]

It should be noted that some grapes, presumably of sport origin, are entirely tetraploid in epidermis as well as internally (reverse of diploid-tetraploid sports now commonly known). The explanation may be that they originated as epidermally tetraploid sports, and later at the growing point of a bud or buds, internal tissue developed from the uppermost layer (L-I), which ordinarily gives rise to the epidermis.[3, 4] Shoots originating from such transformed growing regions would be completely tetraploid and be of sport origin.

In four plants (one of a variety and three of separate selections) a branch of each bore leaves, all with certain deformities. In the plants of the selections, some internal tissue was presumably involved, thus causing leaf deformity. However, in one plant of the Scuppernong variety, the epidermis alone was affected. The leaves on a branch had a hairy appearance and the epidermis proved to be tetraploid. In contrast, tetraploidy in the epidermis in other plants did not show a similar effect. These peculiarities appearing in grapes

FRUIT AND SEED SIZE
Figure 15

← *A* and *C*—Fruits were taken from one area in each cluster shown in Figure 13. *A* is diploid and *C* is tetraploid. These clearly show larger berry size in the tetraploid group. *B* and *D*—Groups of seeds shown in respective rows and order from fruits shown in *A* and *C*. In this sample, the tetraploid seeds are more round and broader than those of the diploid which are smaller and in some cases are defective. (All × 1.) *Photographed by Robert L. Taylor, Sr.*

following colchicine treatment may be due either to some genic disturbance or to aneuploidy in some part of the tissue of the branches and leaves as a result of irregular chromosome distribution. The periclinal nature of these peculiarly developing branches was recognized from the fact that the epidermis in some was diploid and in others tetraploid. Some lateral branches appeared to be normal. In plants where the leaves appeared distorted, presumably L-II as well as L-III was affected,[3,4] but L-III was probably normal since some portion of the center of the leaves was normal green and smooth, and the marginal portion wrinkled and of a lighter shade of green. A careful cytohistological study of these aberrant forms will have to be made to determine their exact nature.

As was pointed out earlier, *Vitis vinifera*, the European type of grape which is mostly grown in California, and *V. labrusca* and other species native to the northeastern United States have $2n = 38$ chromosomes (Nebel,[9], Sax[11]); *V. rotundifolia*, the muscadine grape of the southern states, has $2n = 40$ chromosomes (Sax[11]). Some hybrids have been obtained between the two types but they have been sterile. There have been many instances in other plants in which chromosome doubling of sterile hybrids has brought about fertility. With the acquisition of tetraploid plants in both chromosomal types, we now have an opportunity to obtain hybrids between the two cytological forms of grapes and to determine whether fertile ones can be obtained. Varieties combining the disease resistance and vigor of the muscadines with the bearing habit of the bunch grapes would be of considerable value.

Summary

Over a two-year period polyploidy was successfully induced by colchicine treatment in 10 varieties and selections of bunch grapes adapted to the northeastern United States, in one *V. vinifera* grape of California and in 16 muscadine grapes adapted to the southern United States. The technique and the identification of tetraploidy is described in detail.

Chromosome doubling has insured fertility in some sterile hybrids in other species. Crosses will be attempted between tetraploids of bunch and muscadine grapes in the hope that fertile hybrids may result from these crosses.

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GENETIC BASIS OF SOME WHITE PHENOTYPES IN THE RANCH MINK

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THE market demands for a clear white pelt have steadily increased for the past few years, and the prices commanded by such pelts today have had a tendency to place the white mink in a class by itself. In retrospect, this situation appears somewhat anomalous to the student of coat color genetics since the gene for albinism was among the first of the mutant genes to be detected in the ranch-bred mink (*Mustela vison* sp.), and consequently could have been tested systematically as a possible starting point for the production of a clear white pelage. Only passing interest was shown in the albino, however, probably as a result of the following circumstances: in combination with an otherwise unselected genome, the color phase produced by homozygosity for this gene is not a clear white, but varies from a near white coat except for some yellowish brown coloration in the hair of the nose and especially the tail, to one that in addition may be creamy white over the entire body. Attention came to be focused on the so-called "dominant" whites, because of their clear snowy whiteness, leading to the neglect of the albino and the subsequent loss of interest in this genotype.

It should be clearly stated at this point that breeders and the fur trade do not necessarily mean that a white mink must be completely without pigmented hairs to be designated as such, but may and often does have "not . . . over twelve dark hairs on their body" as stated by Osborn.[4] Most significance is attached to the clearness of the white in areas that are free from pigmented hairs, provided that the colored spots do not account for more than about five percent of the usable parts of the pelt.

Historical

Noble[3] appears to have been the first to call attention to the white mink subsequent to the recognition of the potential value of the various mutant color phases in this species of fur bearer by commercial breeders. Smith, *et al.*,[7] presented an analysis of the breeding records of the mink mentioned earlier by Noble, named the color phase silver, and symbolized the mutant gene by an *S*. These authors state: "The white spotted and silver offspring which resulted from the mating of this original male with wild type females have varied much in the extent of white spotting and in silvering." It is interesting to note that after more than a decade of breeding experiences with *S* in myriads of genetic combinations, Smith at this early date placed all mutant type offspring from silver $\times$ silver (presumably $Ss \times Ss$) matings in a single class, prophetically indicating that "Judgment as to the nature of the homozygous dominant silver mink must be withheld until a greater number of matings is made."

All *SS* genotypes are now known to fall in the "95 percent white" phenotypic classification, although mink heterozygous for *S* may be less pigmented than some *SS* individuals, depending upon the residual inheritance. The color phase regularly produced by *S* when singly substituted in an unselected background has come to be known as the black cross by most mink breeders, and all pelts of this phenotype marketed by the Mutation Mink Breeders Association

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bear the trademarked name Royal Kohinur.

Osborn[4] reported in 1945 that he had obtained some "nearly one hundred percent basically white mink" from black cross (*ffSs*) × silver sable (*Ffss*) matings. Castle and Moore[1] discussed some of the effects of the gene for black cross in homozygous condition, and in combination with the gene for silver sable which they have designated by *F*; in this same paper c^H is employed to symbolize the gene for albinism. Cole and Shackelford[2] noted that mink of the genotype *FfSs* are "dominant white," and are phenotypically indistinguishable from *SS* individuals.

Production Difficulties

Several clear-cut arguments have been advanced to demonstrate the relative inefficiency in breeding the basic genotypes most commonly used to meet the requirements of the fur trade for a white mink pelt. Some of the most important may be grouped into three categories: (1) the males of one genotype are usually sterile, and in another whiteness is a result of simultaneous heterozygosity for two dominant genes; (2) certain genotypes are reported by breeders to be "poor producers"; (3) some genotypes have either pigmented guard hairs at the base of the tail and on the head, or a sparse scattering of pigmented guard hairs over the entire body. The genotypes most often encountered on commercial ranches may be considered in the light of the points listed above, and six of them will be mentioned here.

Prior to the 1952 pelting season, the major portion of the white pelts in the fur trade were taken from "dominant" white mink of either the *FfSs* or *ffSS* constitution. Females of these genotypes are invariably classed as poor producers by their owners, on the average, although no genetic basis is readily apparent in support of this contention and it is possible that the fault could be overcome by selection. It is believed by some that *FF* individuals fail to survive, and there is considerable evidence that such mink are less viable than *Ff* or *ff* mink; this could account for poor production in *Ff* × *Ff* type matings which may represent a considerable portion of all white by white matings since the two genotypes *FfSs* and *ffSS* are phenotypically indistinguishable as has already been mentioned. Except for *SS* × *SS* matings, varying proportions of low commercial value nonwhite color phases result from matings of white males of either genotype to females of any genotype; this alone could make the difference in profit or loss on some ranches.

The average white mink of either the *FfSs* or *ffSS* genotype has a small area of pigmented hairs on the top of the head and at the base of the tail. As has been indicated above, at the present time this is not considered too great a fault, but it is desirable to eliminate such areas when possible. Attempts to diminish the contrast between the pigmented and white areas have been successful following the substitution of several of the recessive mutant genes; the so-called "white sapphires" (*alalppFfSs* or *alalppSS*) fall into this group. It is doubtful, however, that the gains made by this genetic technique are commensurate with the time and effort expended.

The genetics of the "Stewart" mink needs considerably more investigation, but the phenotypic effects of this factor has led Shackelford[6] to consider the possibility that it is identical with the factor for silver sable (*F*). This problem should be included in this discussion since a clear white mink with wild type eye color is found occasionally among the "homozygous Stewart" kits resulting from "Stewart" × "Stewart" matings. Two major difficulties have been experienced by breeders of this type white mink. (1) "Homozygous Stewart" males have been reported to be sterile, and a single individual kept on our experimental ranch for two seasons produced no offspring after mating in a normal manner with more than a dozen females. Although females of this genotype are known to have produced kits, there seems little doubt that fertility is reduced. Most breeders consider all such females to be sterile and routinely pelt them. (2) The proportion of the "homozygous Stewart" individuals that pass for whites varies considerably, presumably as a result of the residual inheritance within a particular herd; in no case known to us, however, were more than half of the mink of this genotype white—the remainder being more or less sprinkled over the dorsal parts of the body with pigmented guard hairs.

A mink simultaneously homozygous for the gene for black cross (*S*) and the gene for albinism (c^H) is a clear white with pink eyes. The greatest difficulty encountered by breeders of this type results from the difficulty in distinguishing between c^Hc^HSS and c^Hc^HSs individuals in some cases, which can lead to the production of mink with considerable amounts of yellowish brown coloring on the head and tail in subsequent generations. Probably a less important objection is that females of this genotype (c^Hc^HSS), along with all other *SS* combinations, are considered to be poor producers. In spite of the difficulties just enumerated, it is possible that the propagation of this genotype may become one of the standard ways of obtaining white pelts.

Clear white pelts can be produced from two other basic genotypes by the selection for specific modifiers. The fur of the average albino mink (c^Hc^H) in certain herds is almost free

of pigmentation, and some individuals produce a pelt that will be accepted as white by the fur trade. The goofus[5] color phase (*oo*) regularly exhibits a pattern similar to that of the siamese cat, but clear white mink of this genotype can result from selection for appropriate modifiers. White pelts from these two genotypes are comparable in quality to those of other genotypes, but the same weakness is shared by both of them as far as the practical fur farmer is concerned: time and effort are required to obtain a breeding herd, and the introduction of either type into an alien herd will usually lead to the reappearance of poorly colored (not clear white) pelts for a minimum of several generations thereafter.

Albino-Pastel (brown eyed) White

Mention was made in the opening paragraph of this paper that the albino color phase was early abandoned by breeders in general as useless for the production of a marketable pelt. As has been the case with most of the recessive mutant genes, however, the gene for albinism occasionally revealed its presence in numerous combinations of mutant genes in herds that were not known to be "contaminated" with c^H. A number of alert breeders have noted that mink of the genotype bbc^Hc^H are completely white, and compare favorably in clearness of color with the best whites produced by other genotypes. Consequently, attempts are being made at this writing to increase the breeding stocks of the albino-pastel as rapidly as possible so that another genetic way of producing white pelts will be available within a few years.

The Hedlund White

What would appear to be one of the most practical answers to the market requirements for high quality self white mink pelts was discovered by Mr. Ernest Hedlund of St. Paul, Minnesota, about 10 years ago. Completely white mink with wild type eye color appeared among the offspring in some of his experimental matings, and these individuals formed the basis for a sizeable group of this color phase. After several years of work in testing the results of combinations involving his white mink with many of the other mutant color phases, Mr. Hedlund disposed of his entire herd. Five breeders within the state of Wisconsin came into possession of all known individuals of the Hedlund type white mink, and the data presented in this paper represent the breeding results on one of these ranches with which one of us (WDM) is associated. Commencing with the 1954 breeding season, this genotype has been made available for purchase by the general public.

Non-white × Hedlund white matings produce only non-white kits as is indicated in Table I. When the resulting non-white kits are mated to whites, approximately half of the offspring are white and half are non-white. White × white matings produce only white kits. It is clear from the evidence presented here that homozygosity for a mutant gene which we choose to symbolize by *h* results in a mink with a clear white pelage and wild type eye color.

THE "HEDLUND" WHITE MINK
Figure 16

Several genotypes are responsible for the self white phenotype in the ranch mink. The "Hedlund" white is one of the most popular types within this color phase, and at present is the largest single source of white pelts in the fur trade.

The increased amounts of white spotting exhibited by the major portion of *Hh* animals usually serves to distinguish such mink from the wild type, indicating that in an otherwise unselected genome *h* is not completely recessive to its allele as has been the case to date for the other recessive mutant genes affecting self coloration in this species. The presence of *h* in approximately 85 percent of *Hh* individuals is revealed by a noticeable increase of the ventral white spotting characteristic of the ranch mink. In addition, a few *Hh* mink are completely white below, have white feet and tail tip, with an occasional individual showing an asymetrical white blaze on the face reminiscent of the Colmira[5] (*Cmcm*) color phase. The observation that certain white males "mark" their non-white offspring more conspicuously than others may be interpreted to mean that specific modifiers influence the penetrance of *h;* if this is true, it is reasonable to assume that the un-

desirable excess white spotting in *Hh* mink can be appropriately diminished by selection. On the other hand, it is possible that the manifestation of these white markings is a result of genetic factors not associated with, nor dependent upon, the presence of *h*.

The behavior pattern of the Hedlund white differs in several respects from the standard ranch mink. It appears to be more docile than the wild type or any other well known color phases, and when handled in the usual manner rarely attempts to bite. Loud noises do not distract it, and when sleeping this white mink can be picked up without difficulty if the pen is not vibrated. This characteristic can best be demonstrated by observing a mixed litter of sleeping kits; when the top of the next box is disturbed the non-white siblings will awaken immediately and leave the box, while the whites will remain asleep.

Breeders of this white type have experienced difficulties in getting these mink to mate and care for the young properly. Males that will copulate usually mate with more females than is ordinarily expected, but a large portion of the females will not accept a male. Those females that do mate produce litters of comparable size to other genotypes (Table I), but prove to be poor mothers by neglecting their offspring. All of these deviations from normal behavior suggest that the Hedlund white mink may be unable to hear.

Summary

Six genotypes producing a white pelage in the ranch mink acceptable for commercial use have been mentioned, and some of the reasons that none of them is presently considered an adequate basis for economical production of white pelts have been enumerated; a seventh, the albino-pastel (*bb*) white, will probably become a standard combination for production of white pelts in the future. The Hedlund white is presently one practical answer to the market requirements, and results from homozygosity for a mutant gene symbolized by *h*. Approximately 85 percent of the mink heterozygous for *h* can be distinguished from the wild type by increased ventral white spotting which occasionally extends to the feet, tail tip, and rarely to a blaze on the face. The observation that certain white individuals "mark" their non-self white offspring more conspicuously than others indicates that the penetrance of *h* may be influenced by specific genetic modifiers. The behavior pattern of the Hedlund white differs in several respects from other mink, suggesting that it may be deaf.

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TABLE I. Breeding results of the Hedlund White*

Parents ♂♂ ♀♀	Offspring White	Non-White	Total	Number Litters	Average Litter Size	Year
White (*hh*) Non-White (*HH*)	0	202	202	40	5.1	1952
	0	118	118	20	5.9	1953
total	0	320				
expected (0 : 1)	0	320				
White (*hh*) Non-White (*Hh*)	231	246	477	101	4.7	1952
	757	788	1545	319	4.8	1953
total	988	1034				
expected (1 : 1)†	1011	1011				
White (*hh*) White (*hh*)	10	0	10	3	3.3	1952
	145	0	145	29	5.0	1953
total	155	0				
expected (1 : 0)	155	0				

*Data obtained from the James Moore Ranch, Peshtigo, Wisconsin.
† $\chi^2 = 1.05$, $P > 0.30$

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THE RESISTANCE TO DDT BY HOUSEFLIES

Some Genetic and Environmental Factors

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THE rate of reproduction is high and the generation turnover requires only a short time in the housefly. Many reports have shown that flies have developed resistance to DDT both in the laboratory and under field conditions. This makes the housefly an excellent animal in which to study the inheritance of resistance, and to determine some of the environmental factors which may influence resistance. This type of study might lead to a clearer understanding of resistance in general and the findings might apply to the development of resistance in economically desirable animals and might offer a way to overcome resistance in the undesirable ones. Therefore, the purpose of this study was to determine the mode of inheritance of resistance to DDT and to determine some of the factors affecting this resistance.

Materials and Methods

For determining resistance, a measured amount of DDT was applied to the thorax of each fly. The insecticide was measured by means of a micrometer and a ¼cc tuberculin syringe. The syringe was equipped with a .27 guage needle bent to 90 degrees and with the point filed off so that the opening came at the end. The amount of DDT in the dose was regulated by the percentage solution used with .001 ml. being delivered at each dosage. The DDT in all tests was in acetone solution. The acetone evaporated quickly, leaving the DDT on the thorax of the fly. All tests were made when the flies were not more than two days old. All tests for comparative purposes were conducted on the same day and as near the same time as possible.

A colony of each of two resistant strains of flies, the Bellflower and the Orlando, and a colony of susceptible ones, were obtained from the U. S. Bureau of Entomology and Plant Quarantine at Corvallis. One line of Orlando was maintained without exposure to DDT or other insecticide for nine generations. The flies of the ninth generation were tested to see if their initial resistance had been maintained. One colony of Bellflower flies was maintained for six generations in a DDT-free environment and the flies of the sixth generation were tested.

Individual and mass matings were made. In the individual matings, F_1 flies were poisoned after the eggs were laid. If a supposedly resistant fly died from the treatment or if a supposedly susceptible one lived after treatment, the eggs from that mating were discarded. Eggs were used only from matings where the resistant parent lived after treatment with 10 micrograms of DDT and the susceptible parent died from a much smaller dose. In the mass mating method several males of one strain were placed with several females of the other. All eggs of these matings were used. Reciprocal crosses were made. Comparisons were made between the resistance exhibited by the F_1 and F_2 of the individual and mass matings. The F_2 in all cases came from inbreeding the F_1 by the mass method. All F_2 flies were counted and treated in order to obtain a ratio as nearly correct as possible.

As soon as it became apparent that the results of the individual matings did not differ materially from those of the

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mass matings, the individual matings were discontinued. Results of these matings up to that time were combined with the mass mating results.

Results

Test for Sex Linkage

Reciprocal crosses were made between Bellflower (resistant) flies and susceptible ones. The F_1 from both types of matings were treated with the same dosage of DDT. There were no significant differences in resistance of flies produced by the two types of matings. Sex linkage was not indicated. (Table I).

Although there appeared to be segregation of resistance in the F_1 which would indicate that one pair of genetic factors was concerned, resistance was generally intermediate between that of resistant and that of susceptible flies. These results are in accord with those of Keiding[4], and of some of those of Harrison.[3] In our F_2 flies, however, we got results sharply at variance with those of Harrison.

Differences in the Survival Percentages of the F_2 Flies

When the F_1 flies from each cross were inbred, the F_2 flies from one cross proved to be much more resistant than those from the reciprocal cross. The F_2 flies produced by inbreeding F_1 flies from resistant females × susceptible males were much more resistant than the F_2 flies produced by inbreeding F_1 flies from susceptible females × resistant males (Table II). This difference was significant as shown by the chi-square test ($P<.01$). These data were secured by using F_2 flies that came from untreated F_1 flies. When F_1 flies that survived treatment with 10 micrograms of DDT were used to produce F_2 flies, there was also a significant difference in survival percentages, depending upon the kind of F_1 flies that were used to produce the F_2 flies (Table II). When the F_1 flies were produced by mating resistant females to susceptible males, the resulting F_2 generation flies possessed more resistance than when the F_2 generation flies were produced from F_1 flies that were developed by mating susceptible females to resistant males (Table II).

TABLE I. Comparison of the survival rates of the F_1 from reciprocal crosses of Bellflower × susceptible flies

Mating	Flies treated Male	Flies treated Female	Dosage of DDT Micrograms	Survival rate Male Percent	Survival rate Female Percent
Resistant male × susceptible female	131	166	5	37	54
Susceptible male × resistant female	712	336	5	40	44
Resistant male × susceptible female	78	80	10	17	34
Susceptible male × resistant female	74	37	10	30	46

Resistance Differences in Backcrosses to Susceptible Flies

When the F_1 flies were backcrossed to susceptible flies, there was a marked difference in the amount of DDT that could be tolerated, depending on how the F_1 were produced. The backcross flies produced by mating F_1 (resistant male × susceptible female) flies to susceptible ones could tolerate only one-tenth the quantity of DDT as backcross flies produced by mating F_1 (susceptible male × resistant female) flies to susceptible ones (Table III).

Resistance Differences in Backcrosses to Resistant Flies

In backcrosses of the F_1 flies to the resistant parent stock, there was a significant difference in the survival rates of the backcross flies depending on how the F_1 flies were produced. When F_1 males from a resistant female × a

TABLE II. Comparison of the survival rates of F_2 flies from reciprocal crosses of Bellflower × susceptible flies

Composition of F_1 used to produce F_2 flies*	Results of tests made on F_2 flies† Flies treated Male	Flies treated Female	Survival rate Male Percent	Survival rate Female Percent
Resistant males × susceptible females F_1 untreated	200	1348	6	5
Susceptible males × resistant females F_1 untreated	303	668	25	23
Resistant males × susceptible females F_1 treated	55	105	11	31
Susceptible males × resistant females F_1 treated	224	169	59	67

*The parents of part of these flies were not treated. The parents of the others were given 10 micrograms of DDT and the survivors were used to produce the flies treated in this test.

†All flies treated in this test got 10 micrograms of DDT.

TABLE III. Resistance of the offspring from backcrosses of the F_1 × susceptible flies

Composition of backcross flies	Results of tests made on backcross flies Flies tested Male	Flies tested Female	Dosage of DDT Micrograms	Survival rate Male Percent	Survival rate Female Percent
F_1 females (resistant males × susceptible females) × susceptible males	283	290	1	16	19
F_1 females (susceptible males × resistant females) × susceptible males	219	29	10	8	21
F_1 males (resistant males × susceptible females) × susceptible females	353	416	1	14	16
F_1 males (susceptible males × resistant females) × susceptible females	174	280	10	3	6

susceptible male were bred back to resistant females, their offspring had a significantly higher survival percentage than the backcross offspring of F_1 males from a resistant male × susceptible females (Table IV). When F_1 females were bred back to resistant males the backcross flies had a resistance in accordance with the way the F_1 flies were produced. If the F_1 flies used in the backcross resulted from crossing resistant females × susceptible males, the resulting backcross flies were more resistant than when the F_1 flies used in making the backcross resulted from crossing susceptible females with resistant males (Table IV).

Resistant females mated to F_1 males produced backcross flies with less resistance than resistant males mated to F_1 females (Table IV). The backcross flies resulting from the crossing of F_1 females with resistant males emerged from the pupae two days later than the ones produced by mating F_1 males to resistant females. This difference in development time of two days may be the cause of the difference in resistance. A test to see if a difference in the time taken for development has any effect on resistance was later made.

Decline in Resistance

In the Orlando strain, resistance declined after nine generations in a DDT-free environment until resistance was little above that of the laboratory susceptible strain. This is in accord with the findings of King.[5] The survivors of this test were interbred and their progeny were tested for resistance. There was a marked increase in resistance in these flies over that of their parents. Selection was involved here but it is possible that selection alone did not account for all the increase in resistance which was noted.

Bellflower flies were tested after six generations in a DDT-free environment. The resistance of these flies was significantly lower than that of first generation flies. However, the decline in resistance was not as marked as was that in the Orlando flies.

These results apparently disagree with those of March and Metcalf[6] with the same strain of flies. This disagreement, however, is more apparent than real because, according to a statement of Dr. March (recorded on page 25 of the "Proceedings of a Conference on Insecticide Resistance and Insect Physiology," National Research Council, 1952) his flies were developed at a temperature of 60°F. Our flies were developed at a temperature above 70°F., and the temperature of the media was considerably above that. A discussion of the relationship of developmental temperature to resistance will be found elsewhere in this paper.

TABLE IV. Resistance in flies produced by backcrossing F_1 flies to the resistant parent stock

Composition of flies	Results of treating flies with DDT*			
	Number treated		Survival rate	
	Male	Female	Male Percent	Female Percent
F_1 males (resistant males × susceptible females) × resistant females	218	236	6	12
F_1 males (susceptible males × resistant females) × resistant females	60	107	13	21
F_1 females (resistant males × susceptible females) × resistant males	82	167	17	23
F_1 females (susceptible males × resistant females) × resistant males	247	262	28	30

*Each fly was treated with 10 micrograms of DDT.

Effect of the Length of Development Time upon Resistance

By means of temperature cabinets one colony of flies was incubated at 90°F. and the other at 70°F. The temperature of the media was actually higher in each case. The media incubated at 90°F. averaged 101°F. and that incubated at 70°F. averaged 79°F. The temperature of the larval media was highest the first two days of larval development and gradually dropped to one or two degrees above incubator temperature by the time of emergence.

In the first two experiments, a summary of which appears in Table V, 32 comparisons were set up. One hundred flies were used in each comparison. Comparisons were made at four different dosages of DDT for both males and females both at three days of age and at four days of age. There was no difference in the mortality rates at three and four days of age, so these comparisons were used as replications in the analyses.

There was a tremendous difference in the mortality rates of flies raised under the two different temperatures (Table V). The cumulative chi-squares of these 32 comparisons amounted to 306.37 with 32 degrees of freedom. Chi-square for the totals was 146.30 with one degree of freedom. These flies were all tested at room temperature.

It is shown in Table V that the flies raised under an incubation temperature of 90°F. emerged as adults seven days earlier than the

TABLE V. Summary of the influence of developmental temperature on DDT resistance of house flies

Developmental Temperature	Length of Developmental Period	Dead Flies 24 Hrs. After Treatment	Live Flies 24 Hrs. After Treatment	Total	χ^2
Degrees	Days				
90	9	1035	565	1600	146.30
70	16	694	906	1600	P = <.01
	Totals	1729	1471	3200	

flies incubated at the lower temperature. It was also noticed at the time of the first tests that the flies emerging later under the 70°F. incubation temperature were larger than those incubated at 90°F. Since this difference in size could have accounted for the difference in resistance in the first series of comparisons, a second series was set up. In the second series all conditions were the same as in the first series except that the dosage of DDT was increased for the flies incubated at 70°F. to compensate for their larger size. This increased dosage was determined by weighing a sample of 30 of the flies developed at 90°F. and the same number of the flies developed at 70°F. to get the average difference in weight. The increased dosage for the flies developed at 70°F. was determined from these average weights.

As may be seen from Table VI, the adjustment of dosage to compensate for size reduced the differences due to the different incubation temperatures and consequently the longer development time to a lower figure but the differences were still highly significant. The cumulative chi-square for the 32 comparisons was 117.49 with 32 degrees of freedom. The chi-square for the totals was 63.52 with one degree of freedom.

The difference in incubation temperature did not make as great a difference in resistance of males as it did in that of females but was highly significant even in the case of males.

Discussion

The loss of resistance in the flies of the Orlando strain may be a manifestation of the phenomenon known as Dauer-modification. A Dauer-modification is defined as "a cytoplasmic change induced by an environmental factor which decreases in penetrance and expressivity in succeeding generations in the absence of the inducing stimulus".[2] The cytoplasmic factor apparently reproduces itself rapidly enough to maintain resistance only in the presence of DDT. When DDT is removed, the resistance decreases until by the ninth generation very little is left.

The Dauer-modification theory, however, falls short of completely explaining resistance in the Orlando strain. Logically, if this theory were the true explanation, all offspring of resistant parents would be expected to have equal resistance unless the resistance material in the cytoplasm is particulate, because resistance seems to range from a very small degree to a very large one in flies of the same hatch with the same breeding.

TABLE VI. Summary of the influence of developmental temperature on DDT resistance of house flies when the dosage of DDT was adjusted for difference in body size

Developmental Temperature	Length of Developmental Period	Dead Flies 24 Hrs. After Treatment	Live Flies 24 Hrs. After Treatment	Total	χ^2
Degrees	Days				
90	9	1187	413	1600	63.52
70	16	976	624	1600	P=<.01
	Totals	2163	1037	3200	

The fact that resistance is greater when transmitted through the female and the fact that there is no segregation in F_2 according to any recognizable genetic pattern of the resistance factor or factors, both point to the conclusion that the resistance factor is carried in the cytoplasm. These results are in accord with those of Bruce.[1]

The fact that resistance lasts at full strength for one generation after being transmitted by the male suggests that there may be interaction between the cytoplasm and one or more pairs of genes. If this is the case, the genes transmitted by the male are apparently conditioned by the male's own cytoplasm to produce resistance in the F_1 flies. Apparently the cytoplasmic factor has to be there for resistance to be maintained, but the effect of this factor on the genes lasts through the next generation.

It has been shown that a small amount of the cytoplasmic substance may be transmitted in the sperm.[2] This may explain why a few of the F_2 flies produced by inbreeding F_1 flies from resistant males $\times$ susceptible females were able to survive a treatment with 10 micrograms of DDT.

Sonneborn[9] has shown that there are specific particles in the cytoplasm of the so-called "killer" strain of *Paramecia* that are responsible for the "killer" character. He has shown that these particles reproduce themselves when a certain gene is present in the nucleus. This nuclear gene which Sonneborn has named "*K*" and the cytoplasmic particles which he has called "kappa" must both be present in the cell for the "killer" character to be perpetuated. Kappa does not necessarily reproduce itself at the same rate as the cell itself divides; nor does it necessarily divide equally between the two daughter cells at mitosis.[8] Although the gene *K* is responsible for kappa reproduction, it cannot initiate production of kappa if there is none present in the cell. Sonneborn and his co-workers have actually seen these kappa particles. They have also been able to reduce or destroy kappa by raising the temperature of the medium in which the animal lives.[10]

It seems reasonable to think that resistance to DDT in the Orlando strain of flies may be due to particles in the cytoplasm which act like Sonneborn's kappa. In this case, however, it appears that DDT itself may be necessary for the reproduction of the particles in addition to a nuclear gene similar to Sonneborn's, particularly if the weather is warm and development is fast.

This nuclear gene may account for reproduction of the kappa-like substance, but it apparently needs the stimulus of DDT in the environment to maintain reproduction of the cytoplasmic substance at a high level. It is certainly true that as soon as DDT was reintroduced into the environment of ninth generation flies there was a marked increase in resistance in the offspring of the flies surviving treatment.

A possible explanation for the decline in resistance may be that the cytoplasmic particles reproduce at a certain rate unless stimulated by DDT, and that, under the conditions of this experiment, this rate was too slow to keep up with the accelerated rate of fly reproduction. This would cause a reduction of resistance. It was noted during the early stages of the experiment that flies emerging from the pupae last appeared to be more resistant that those emerging first. This phenomenon was also noticed by Pimentel *et al.*[7] It was not known whether this longer time in the development stages caused the production of the resistance particles to keep up with the reproduction of the flies, which in turn caused the flies to exhibit more resistance, or whether resistance itself caused a slower process of development. It was thought that if environmental conditions were altered so that the process of development was slowed down, resistance might be maintained.

It has been established conclusively that a relationship exists between incubation temperature and the resistance of the LDD strain of houseflies to the toxic effects of DDT. The higher the temperature during development, the shorter was the period of development from egg to adult. The shorter the development period, the less resistant are the flies.

It may be that the extra seven days' time could account for the difference in resistance, if this extra time allowed the reproduction of the resistance particles to keep up with the reproduction of the flies. If this were true, however, it would be expected that the flies would become more resistant as they get older. This is not the case.

Another explanation is that the extra heat, within itself, is the cause of lowered resistance. The heat may alter or destroy the resistance particles present in the cells.

The possibility of interaction between both time and temperature should not be overlooked. A longer time in development and a lower temperature may both be necessary to bring about the extra resistance noted in the flies raised at the lower temperature. Further research is needed.

The Bellflower strain of flies may not have exactly the same sort of resistance as that exhibited by the Orlando strain. Their decline in resistance was much slower than that of the Orlando strain. It was noted during the experiment that the flies of this strain emerged from the pupae from two to four days later than the flies of the Orlando strain. The eggs were laid the same day and were incubated under the same conditions. This fact suggests that the kind of resistance may be the same and that the theory of the speed of reproduction of flies being greater than the speed of reproduction of the resistance particles might be the correct answer. In this strain, also, the last flies to emerge appeared to be more resistant than the first.

March and Metcalf[6] had no decline in the resistance in their Bellflower strain in 30 generations. The flies used in this experiment may have been slightly different genetically. However, March and Metcalf treated their flies at a greater age (two to four days). They also raised their flies at 60°F. In the work of March and Metcalf the production of the resistance substance may have had time to keep up with the reproduction of the flies.

The offspring of the surviving Bellflower flies treated in the sixth generation showed a significant increase in survival percentage over the flies of the sixth generation. This may or may not have resulted from selection alone.

It is suggested that the DDT itself acted as a stimulus to the production of the cytoplasmic substance just as it did in the Orlando strain and that the resistance in the two strains is the same. Other factors account for the difference in the time required to go from egg to adult in the two strains and this time difference in turn accounts for the difference in the rates of decline in resistance. The time for development in both strains was cut as short as possible in this experiment and this factor alone may have caused the decline in resistance in both strains of experimental flies. It is suggested that with a rate of development which is slow enough, there would be no decline in resistance in either strain.

Conclusions

No evidence for sex-linkage of resistance to DDT could be found.

The factor responsible for resistance to DDT in Bellflower and Orlando houseflies apparently is carried in the cytoplasm of their cells.

The factor responsible for DDT resistance is particulate. No alternate conclusion can be drawn from the data obtained in this experiment.

It seems reasonable to assume that the reproduction of the cytoplasmic resistance particles is under the control of one or more nuclear genes. This is indicated by the fact that there is a one generation lag in the virtual disappearance of resistance when all of the resistance comes from a resistant grandfather.

When DDT is introduced into the environment of DDT resistant houseflies in which resistance has declined, the offspring of these treated flies have much more resistance than the parents. Part of this added resistance is accounted for by selection. The remainder of

the increase in resistance may be due to a stimulating effect of DDT on the reproduction rate of the resistance particles or to the activation of new particles by the DDT.

The speed of development from egg to adult influences resistance in some strains of DDT resistant houseflies. The longer the development period the greater the resistance, within reasonable limits.

A few F_2 flies, in which resistance comes from one resistant grandfather, are fully resistant. This indicates that a few of the cytoplasmic resistance particles may be transmitted in the sperm by the resistant male.

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Liturgical Color or Race Crossing?

To the Editor:

Some months ago the Journal carried an interesting sketch with a reproduction of a panel painting in the Calw, Germany. We have used both the Journal and Dr. Stern's textbook here, as we can state, with pleasure. However, artistic criticism and biological interpretation are at times difficult matters to harmonize. You might be interested to know that we gave our students here, in our periodical in mimeo-style, an alternative interpretation as follows:

> A recent article from the Journal of Heredity gives us paradise as imagined by some artist, unknown, of about 1730. The two authors, Curt Stern and Gertrud Belar, have produced an interesting article conjecturing that an unknown painter piously contemplating on origin of race decided to paint Eve black, and thus left a noble reminder of the tolerance of the eighteenth century. Such a conclusion does not necessarily follow.
>
> The Renaissance gave rise to liturgical interpretation of color. Purple betokened penitence; red, zeal for faith, ardent love or even cruelty and blood-guilt; white, purity, joy and life. Unconscious race unfairness is seen in black for sorrow, despair, death. A cynical misogynist this pious unknown artist may have been, nagging woman as being cause of all the old Adamic alibi blames her with. Black Virgin and Child, not unknown in religious art, we know indicates a deep, divine sorrow. In India, Krishna and Rama in similar role of sorrow, may appear as black.
>
> Liturgical form may be even more puzzling. Man, lion, ox, and eagle were symbols used for the four evangelists. Stodgy peasantry in Europe thought three of the evangelists were factual beast and bird. A benign dragon of the Chinese gives Westerners pause. D. J. Fleming's "Christian Symbols in a World Community" is good beginning material here.

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HYBRIDIZATION OF THE PHEASANT AND FOWL

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HYBRIDS between pheasants and chickens attracted attention in recent years when a commercial firm produced a bird said to have a pheasant as one of its great-great-grandsires, the other great-great-grandparents being chickens. Several investigators have reported that hybrids between pheasants and chickens are completely sterile, and, if this is true, it would be impossible to produce, beyond the first generation, birds which have both pheasant and chicken ancestors. In response to numerous inquiries, an experiment was initiated designed to produce additional information on the pheasant-chicken cross. The pheasant males used in the crosses were produced by crossing Mongolian males with Ringneck females. The chickens used in the crosses were Dark Cornish and Silver Cornish females.

Review of the Literature

The literature on pheasant-chicken hybrids has been adequately reviewed by Suchetet, Poll, Sandnes and Landauer, Quinn, and Yamashina[25, 21, 24, 22, 29] and will not be repeated here except in discussion of various aspects.

Materials and Methods

The pheasant males used in the present experiments were produced by crossing Mongolian (*Phasianus colchicus mongolicus*) pheasant males with Chinese Ringneck (*Phasianus colchicus torquatus*) pheasant females. Males of this type were said to have been mated to Dark Cornish females to produce the commercial pheasant-chicken already mentioned. In the experiment described herein Dark Cornish and Silver Cornish females were used. The Dark Cornish females were developed for superior meat quality characteristics and the Silver Cornish females were being developed for both meat and egg production. The pheasant males were from a Mongolian male × Chinese Ringneck female cross and were about six months of age when received at Beltsville in December, 1950.†

In the spring of 1951 four of the pheasant males described above were placed with 23 Dark Cornish females in breeding pens provided with wire covered yards. Semen for artificial insemination was obtained from the other 10 pheasant males which were kept in exhibition coops within a large laboratory building. Although considerable window space furnished natural daylight, continuous supplementary artificial lights were applied in an attempt to increase semen production. By artificial insemination these 10 males were mated to 44 Dark Cornish and to 16 Silver Cornish females. In 1952, three pheasant males were in a breeding pen with six Dark Cornish females and seven pheasant males were available to furnish semen for artificial insemination of 17 Dark Cornish females.

For the purpose of this study, only the 507 eggs laid during the 10-day period beginning the second day after each insemination were arbitrarily considered as having a good chance of being fertilized. All 902 eggs from floor matings were assumed to have had a good chance of being fertile.

In order to increase the number of hens inseminated semen dilution was employed. Approximately one-third of the inseminations were made with pure

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†Four pheasant males were supplied by Mr. James H. Knowles, Centralia, Washington, and 10 pheasant males were furnished by the Michigan Department of Conservation.

semen and two-thirds with diluted semen. Semen was diluted by mixing one volume of semen with two volumes of modified Ringer solution. A dilution using 30 percent semen and 70 percent modified Ringer solution was found by Bonnier and Trulsson[4] to produce fully as good fertility in fowls as pure semen.

Each insemination consisted of 0.1 cc of diluted or undiluted semen. Artificial insemination was performed up to three times per week. Fertility was determined by breaking the eggs after 7 days of incubation and examining the germ spot for signs of development, except when candling showed the presence of a living embryo at that time.

During 1951 the eggs were incubated at weekly intervals; thus they were set from one to seven days after being laid. During 1952 the eggs were set at more frequent intervals on the assumption that the embryos would have a better chance of developing if they were not stored for as long a period of time before incubating.

Results and Discussion

Fertility and Hatchability

The volume of semen obtained from the pheasant males was quite small, rarely amounting to more than 0.1 cc per male at any one time. However, the small amount of semen obtained was thick and milky in appearance and microscopic examination revealed large numbers of highly motile sperm.

The record of "fertility"* and hatchability from artificial insemination and natural matings of the cross between pheasant males and Cornish females is presented in Table I. Thus, from 106 hens, a total of 1,409 eggs which were assumed to have a good chance of being fertile were incubated and examined for embryonic development. The "fertility" of these eggs was 3.48 percent and the hatchability of "fertile" eggs was 6.12 percent.

Regarding the possible effect on fertility from crossing the two species of pheasants, Blouch[2] stated that they had no evidence to indicate that the Mongolian male × Ringneck female cross is significantly less fertile than purebred pheasants, and in general they had observed the purebred Mongolian and Mongolian × Ringneck crosses come into breeding condition a little later than purebred Ringnecks. This may explain, at least in part, the fact that two hybrids hatched in July and August of the 1951 breeding season.

Early in these experiments Olsen[18] concluded that the early embryonic development of the dead germs was abnormal. Clearly defined embryos were rarely found, yet, upon sectioning these areas of development, embryonic cells were found, indicating that infections or other artifacts were not being mistaken for embryonic development. It will be noted from the summary in Table II that two of the 12 hens which produced "fertile" eggs accounted for 63 percent of the total. The remaining 18 "fertile" eggs were produced by 10 hens.

Some of the "fertility" of the crosses between pheasants and chickens reported here is thought to be due to forces other than true fertilization by sperm. Kosin[14] stated that about 15 percent of the eggs laid by virgin or non-mated Barred Rock and White Leghorn hens contained evidence of abortive parthenogenesis. Olsen and Marsden[20] reported the occurrence of embryonic development in 16.7 percent of the eggs laid by Beltsville Small White turkey hens after being separated from males for 54 to 224 days, and Olsen[19] observed a similar type of development in eggs laid by some of the Cornish females used in this investigation.

TABLE I. "Fertility" and hatchability of eggs produced by Cornish females mated naturally and artificially to pheasant males. (The figures given for artificial insemination are based upon the 10-day period from 2 to 11 days following an insemination.)

Date	Type of mating	Variety of Cornish	No. of hens	No. of artificial inseminations	No. of eggs	Percent "fertility"	Percent hatchability of "fertile" eggs
3-16-51 to 8-30-51	Natural	Dark	23	—	679	2.36	12.50
" " 7-16-51	Artificial	Dark	44	126	96	3.12	0.00
" " "	Artificial	Silver	16	26	78	3.85	33.33
	Total		83	152	853	2.58	13.64
3-22-52 to 7-14-52	Artificial	Dark	17	97	333	1.50	0.00
" " "	Natural	Dark	6	—	223	9.87	0.00
	Total		23	97	556	4.86	0.00
	Grand Total		106	249	1409	3.48	6.12

*An undetermined number of the eggs labeled "fertile" are thought to be the result of development caused by forces other than fertilization by sperm. Thus, the true fertility of the eggs described here is expected to be somewhat lower than the figures indicate.

PHEASANT-CHICKEN HYBRID
Figure 17

A female pheasant-chicken hybrid at three days of age.

It will be noted in Table III that three of the 124 eggs laid by Silver Cornish females in contact with pheasant-chicken hybrid males were found to exhibit early development. Yet, observations made from time to time showed an absence of sexual activity by the hybrid males. All three of these eggs were laid by the same hen, and a year later she was found to be producing eggs capable of early development, although she had been isolated from males during that period. It is suspected that an undetermined percentage of the eggs which began development were not fertilized and that development was due to causes other than sperm. Therefore, the "fertility" figures given in all three tables are thought to be overestimates of the actual amount of fertility produced by pheasant sperm.

Approximately one-third of the inseminations were made with pure semen, but all three of the hybrids produced by artificial insemination were produced with diluted semen.

Incubation Period

All five of the hybrids which pipped, did so on the 25th day of incubation, and the three which were able to hatch were removed from the incubator the following day. Of the two which were unable to hatch, one was helped out of the shell but died a week later.

The incubation period of approximately 26 days is longer than the 21 to 22 day period of chickens and the 23 to 25 day period of pheasants. Wheeler[27] previously reported the incubation period of a chicken-pheasant hybrid as 24 days when the egg was set under a hen.

Sex Ratio

Four of the five hybrids which developed far enough for sexing purposes were males and the other hybrid was a female.

A summary of the results of Guyer, Poll, Cutler, and Kosin[11, 21, 7, 15] in which pheasant males were mated to chicken females reveals 29 male and four female offspring. On the other hand, when chicken males are mated to pheasant females, or in reciprocal crosses, 84 males and 85 females have been recorded by Sandnes and Landauer, Yamashina, and Gowe.[24, 29, 10] Thus, it would appear that pheasant males mated to chicken females produce an excess of males, whereas the reciprocal cross does not.

Behavior of Hybrid Chicks

The hybrid chicks were quite vigorous and were somewhat pheasant-like in that when disturbed they crouched more and darted about with more speed than the baby chicks with which they were quartered. Their call was more like a pheasant chick than a chicken.

TABLE II. Record of "fertile" eggs laid by Cornish hens mated naturally and by artificial insemination to pheasant males

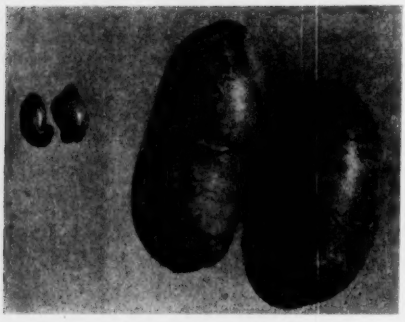
	Hen	No. of eggs	No. of "fertile" eggs	No. of hybrids
1951	1620	55	14	—
(natural)	1600	32	1	1
	1546	28	1	1
		115	16	2
1951	1602	13	1	—
(artifi-	1753	12	2	1
cial)	1761	5	1	1*
	1595	3	2	—
		33	6	2
1952	4313	52	17	—
(natural)	4305	48	4	—
		100	21	—
1952	4320	13	3	1*
(artifi-	4286	5	1	—
cial)	4308	18	2	—
		36	6	1

*These hybrids were able to pip the shell but were unable to hatch unaided.

TABLE III. Eggs laid by Silver Cornish hens confined with pheasant-chicken hybrids from 5-19-52 to 8-11-52

Hen No.	No. of eggs	No. exhibiting development
4403	2	—
4420	37	—
4430	22	—
4448	20	3*
4464	22	—
4497	21	—
	124	3

*The hen which produced these three eggs was later found to be producing eggs capable of some form of development, although she had not been mated for more than six months.



HYBRID AND NORMAL TESTES
Figure 18

Testes of adult pheasant-chicken hybrid (left) and Cornish (right).

Subsequent behavior tended toward more shyness and wildness than noted in the chicks.

Wheeler[27] reported that the call of a pheasant-chicken hybrid chick more nearly resembled pheasant chicks than bantam chicks, and in spite of being fed and watered three or four times daily it grew very wild.

Appearance of Hybrid Chicks

The head of each hybrid at hatching time appeared to be smaller dorso-ventrally and laterally, but larger cephalo-caudad than that of chicks, (Figure 17). The beak and nostrils appeared to be larger than those of chicks. A comb was lacking. The tarso-metatarsal bones appeared thinner and more delicate than comparable bones of chicks. The shanks were slate colored. The down color was predominantly buff, the under parts being lighter than the back, head and neck. A large black dorsal stripe extended from the beak to the tail. This stripe was flanked by one short stripe on each side. The down color was similar to that of Ringneck pheasant chicks as described by Bruckner.[5] Dark Cornish chicks have a similar down color. One of the two hybrids which were unable to hatch was affected with bilateral micropthalmia and the other had a cerebral hernia and a deformed hock. Dissection of these two hybrids revealed normal appearing testes. Testes from one of the hybrids were sectioned and examined microscopically, but could not be distinguished from normal baby chick testes.

Wheeler[27] reported that the color of a pheasant-chicken hybrid chick more nearly resembled Ringneck pheasant chicks than bantam chicks, whereas Yamashina[29] reported the chick feather pattern of the hybrid between a pheasant and Red-backed domestic fowl was intermediate between that of the hybrid pheasant and the Red Jungle fowl.

Adult Behavior

The three living hybrids were easily reared to physical maturity, but were never observed to mate and showed no other signs of sexual maturity. The female became more or less docile as she grew older but the two males remained wild.

None of the hybrids observed by Angus, Henslow, Suchetet or Wheeler,[1, 12, 26, 27] showed any sexual behavior as adults.

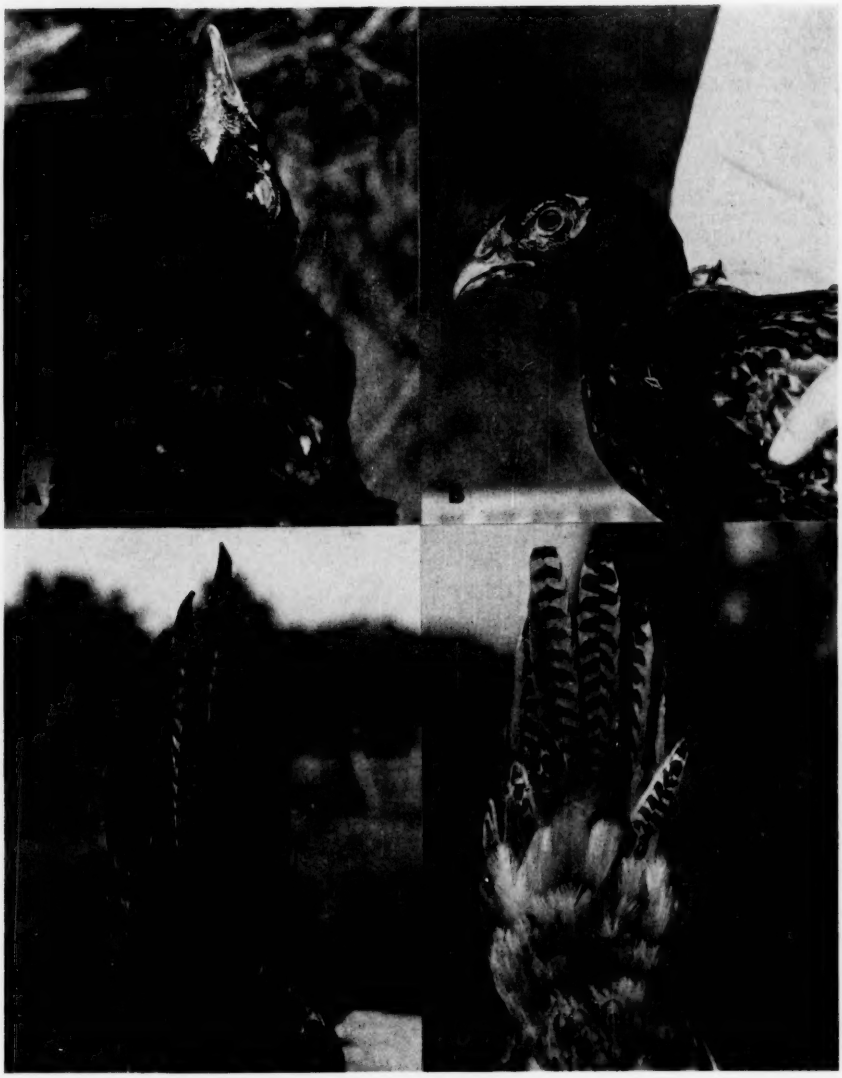
Female Reproductive Apparatus

One of the hybrids in the present studies proved to be a female, and had not produced any eggs when posted at 462 days of age. Dissection showed that she was quite incapable of producing eggs as only a trace of ovarian tissue was found, much less than in a normal, day-old, female baby chick. The oviduct apparently was normally attached to the cloaca. However, it was quite short and weighed only 1.16 grams whereas according to Riley and Fraps[23] the oviducts of normal mature Rhode Island Red females weighed 45.3 grams.

Suchetet[25] reviewed the literature of some early observations of chicken-pheasant hybrids and cited two workers as stating that the eggs are never hatched and that "no fertile eggs were obtained." Dissections of female hybrids reported by Henslow, Poll, and Danforth and Sandnes[12, 21, 8] have invariably revealed degenerated or rudimentary ovaries and abnormal or juvenile oviducts. Kosin[15] stated that a female pheasant-chicken hybrid which he observed never laid an egg.

Male Reproductive Apparatus

The two hybrid males were examined at intervals and attempts were made to express semen by the method of Burrows and Quinn[6] with negative results. Silver Cornish females were placed with the two hybrid males from May 19 to August 11, 1952, and no sexual activity was observed in the hybrids. Three of the 124 eggs laid by these hens showed evidences of embryonic development, and at first it was thought that the hybrids may have mated. However, all three eggs were laid by the same hen, and this hen has since been found to produce eggs showing development caused by forces other than fertilization by sperm. Therefore, it may be assumed that the hybrid males had not mated or were incapable of producing viable sperm. Dissection of the male hybrids revealed small testes which weighed only 0.25 grams in the case of each bird, whereas the testes of a Silver Cornish male of approximately the same age weighed 26.2 grams (Figure 18). The testes of the hybrids were sectioned but no spermatozoa could be found. Male hybrids reported by Blyth, Suchetet, Gowe, and Kosin[3, 25, 10, 15] appeared to be incapable of reproduction, and dissections by Henslow, Angus, Suchetet, Poll, Cutler, and Gowe[12, 1, 26, 21, 7, 10] invariably revealed



HEAD AND TAIL FEATHERS

Figure 19

A—Dorsal view of the head of an adult male pheasant-chicken hybrid. *B*—Side view of the head of an adult male pheasant-chicken hybrid. *C*—Dorsal view of the tail of an adult male pheasant-chicken hybrid. The hybrid's dam was Dark Cornish. *D*—Dorsal view of the tail of an adult female pheasant-chicken hybrid. The hybrid's dam was Silver Cornish.

rudimentary or abnormal testes. Microscopic examination of hybrid testes by Suchetet[26] and Cutler[7] revealed an absence of spermatozoa. Danforth and Sandnes[8] reported that in male hybrids no true sickle feathers were present, saddle feathers were even less fringed than in an ordinary pheasant, and castration seemed to have little or no effect on the form or color of the plumage. These facts indicate the testes of the hybrids were non-functional. Yamashina[29] injected two adult male hybrids with male sex hormone which caused a reddening of the bare parts of the faces but showed no influence on the activity of the germ cells.

Two published accounts are available in which it is implied that fertile pheasant-chicken hybrids have been produced. Both reports lack critical objectivity. One is the report of Fuller[9] on two birds which were midway between pheasants and barnyard fowl in habits, manner and appearance. Mr. Fuller's gamekeeper had certified that the sire of these birds was a pheasant and that the dam was a pheasant-chicken hybrid. The other account is that of White[28] who related that the Irish Dominique Game Fowl resulted when an Irishman tied his game hens out in the bog and allowed the bog pheasant males to mate with them.

Configuration

The general shape of the heads and beaks of the hybrids as adults was more like that of pheasants than of chickens. The body configuration of the female hybrid was more feminine than that of the male, having a relatively larger abdomen and more delicate and refined head and bone structure.

The general shape of pheasant-chicken hybrids has been reported by Angus, Wheeler, Poll, and Lowe,[1, 27, 21, 16] to resemble the pheasant more than the chicken.

Body Weight

The female hybrid produced in the present study weighed 1,776 grams when she was posted at 462 days of age. Body weights of the two hybrid males were 2,200 grams at 418 days of age and 2,140 grams at 383 days, respectively. McAtee[17] reported that, at similar ages, pheasant males and females weigh approximately 1,280 and 900 grams, respectively. Dark Cornish males and females weigh approximately 3,680 and 2,950 grams, respectively, according to Jull.[13] Thus, the hybrids obtained in the present experiments were approximately intermediate in weight between the two parent stocks.

Body weights of pheasant × chicken hybrids have been reported as 1,644 grams by Angus,[1] and 1,446 grams by Wheeler.[28] Poll[21] stated that hybrids usually surpassed both parent forms in weight whereas Lowe[16] stated the hybrids were a little larger than a cock pheasant.

Plumage Color

The adult plumage color of the two male hybrids was predominantly dark (Figure 19*C*) and in general resembled the pheasant except for the characteristic white ring around the neck of the male pheasant. On the other hand, the adult plumage of the female hybrid was predominantly buff (Figure 19*D*). It should be recalled that the female was the offspring of a Silver Cornish, rather than a Dark Cornish female, which may explain the plumage color of one hybrid being different from that of the other two.

The general adult plumage color of hybrids was reported by Wheeler,[27] to resemble the pheasant more than the chicken, whereas Poll[21] and Cutler[7] found them to resemble chickens and Yamashina[29] found them intermediate. The white neck ring of the pheasant was not present in any of the hybrids known to Poll[21] and was not evident in any of the photographs of hybrids presented by Danforth and Sandnes, Yamashina, and Gowe.[8, 29, 10]

Tail Barring

The barring in the tail feathers of hybrids and of pheasant males was of interest (Figure 19*C* and *D*). The black bars in the hybrid tail feathers were wider and the inter-bar spaces were narrower than in corresponding feathers from pheasant males. The tail bars of the hybrids were often irregular and less distinct than in the pheasant male.

Wheeler[27] reported that the tail of a pheasant-chicken hybrid did not show the peculiar color and definite barring characteristics of the tail of the pheasant sire and no tail barring was evident in the photographs of Ringneck pheasant × Leghorn chicken hybrids presented by Danforth and Sandnes.[8] However, Angus[1] reported a hybrid in which the tail barring resembled that of the pheasant, and some of the hybrids pictured by Yamashina[29] exhibited tail barring similar to the hybrids in the present experiments.

Carriage

The carriage of the tail feathers in the adult hybrids was intermediate between the parents. The feathers were spread out more than the pheasant but less than the chicken. The hybrids had none of the bending sickle feathers of the chicken parent.

Wheeler[27] reported that the longest tail feathers of a chicken-pheasant hybrid were broad and rounded at the tip, less long and tapering, carried in a more erect position, and showed less tendency to trail than those of the pheasant sire, and Poll[21] reported that the form and carriage of the hybrid is pheasant-like. Photographs presented by Danforth and Sandnes, Yamashina, and Gowe[8, 29, 10] showed hybrids whose tail feathers were pheasant-like in carriage, but were less tapering than those

of pheasants. No bending sickle feathers were present.

Feather Lengths

The tail feathers in the hybrids of this present study were longer than those of chickens, but were not as long as those of pheasant males. The longest tail feather of each male hybrid was 330 and 343 millimeters, respectively. These may be compared to 558 millimeters for a pheasant male and 216 millimeters for a Dark Cornish male. The longest tail feather of the female hybrid was 254 millimeters.

Angus[1] and Wheeler[27] reported hybrid tail feather lengths of 324 and 213 millimeters, respectively. Poll[21] reported that the tail of the hybrid never reaches the length of pheasant tail feathers. Photographs presented by Danforth and Sandnes, Yamashina, and Gowe[8, 29, 10] give the appearance of tail lengths intermediate between pheasants and chickens.

Head Characteristics

The comb, wattles, and earlobes of the chicken parent were absent in the hybrids obtained in these experiments (Figure 19*B*). The absence of a comb is also shown in Figure 19*A*. During the breeding season, the pheasant male possesses well defined ear tufts and areas of velvety red feathers on either side of the head, but these characteristics were absent in the hybrids.

Wheeler[27] reported that the comb of a pheasant-chicken hybrid was very low, having somewhat the appearance of a rose comb, but without the spike. The wattles and earlobes were absent and the hybrid lacked the velvety feathers on the face which were prominent on the pheasant. Poll[21] reported that there is no semblance of comb and wattles. Photographs presented by Danforth and Sandnes, Yamashina, and Gowe[8, 29, 10] revealed no combs, wattles, earlobes, nor ear tufts.

Shank Color

The shank color was slate like that of the pheasant parents.

Angus[1] reported the shank color of a pheasant-chicken hybrid as brownish gray with the feet darker, whereas Wheeler[27] stated the shank color was intermediate between the two parents.

Spurs

The hybrids' shanks had small spurs which, in size, form and location were intermediate between those of pheasants and chickens. The spurs of the chicken are located slightly to the rear of the inner-side of the shank whereas the spurs of pheasants project almost directly to the rear. The spurs of chickens are long with a relatively small base, whereas the spurs of pheasants are short with a relatively large base.

Poll[21] concluded from a review of the literature that the shanks of pheasant-chicken hybrids are spurless, but Angus[1] and Wheeler[27] reported the presence of spurs in their hybrids, and in a photograph by Gowe[10] a small spur was evident in a location intermediate between that typical of pheasants and chickens.

Summary

Pheasant males produced by crossing Mongolian pheasant males with Ringneck pheasant females were mated naturally and by artificial insemination with Dark Cornish and Silver Cornish females. Among 1,409 eggs, 3.48 percent exhibited development when incubated. The hatchability of these "fertile" eggs was 6.12 percent. One female and four male hybrids developed sufficiently to pip the shell. Two chicks died in the shell and both proved to be males. The remaining female and two male hybrids hatched and were raised to maturity. The incubation period was 26 days. The behavior and general appearance was more pheasant-like than chicken-like. Body weight and tail feather length were intermediate between the parent species. The hybrids had none of the comb, wattle or earlobe characteristics of the chicken parent. The ear tufts and velvety red feathered areas on the sides of the face of the pheasant sire were also absent. The shank color was slate like that of pheasants, and the spurs were intermediate in shape and location between the parent species. No sexual activity was observed and subsequent dissections showed infantile or degenerate gonads.

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◆

The Tortoise Shell House Mouse

(*Continued from page* 158)

to a C57BL/6J male. Tortoise shell females were found in the F_1 generation. This helps to confirm that the gene is dominant and *To* is suggested as its symbol. When piebald spotting (*ss*) is present (it is in the obese stock), both the piebald and the tortoise shell retain their distinctive patterns.

The gene, at present, seems to produce three effects on the tortoise females. First is the tortoise pattern, second is the silkier texture of the hair and slight waving of the vibrissae, and third the skeletal abnormalities in the fore and hind limbs of some of the tortoise females.

Since all tortoise females produced normal as well as tortoise female offspring, it is evident that all these females were of the genotype *Toto*. Presumably it is impossible to produce *ToTo* females because of the absence of *To* males. The small skeletal defects noted in some of the tortoise shell females suggest that the lethality of the gene in males may be due to one or more major skeletal defects.

Linkage studies have not been completed.

TABLE I. Breeding data of tortoise shell females with normal sibs and an outcross to C57BL/6J

	To♀	*to*♀	*To*♂	*to*♂	
7 *Toto*♀ × *toto*♂ (sibs)	33	67	0	32	
3 *Toto*♀ × *toto*♂ (C57BL/6)	20	29	0	13	
Totals	53	96	0	45	194

Summary

Tortoise shell, a new mutant in the house mouse, occurred in the obese stock. It resembles the tortoise shell cat. Breeding data indicate the mutant is dominant and that it appears to be a sex-linked lethal since there is a deficiency of male offspring. *To* is suggested as the symbol for the character.

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PLATO'S GENETIC THEORY

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IT was Plato (427-347 B.C.) who first realized the tremendous potential implications of genetics for social planning; and eugenics was his invention. A great deal of material has been written about Plato's treatment of eugenics in the *Republic*. For the most part, these discussions do not take sufficient account of the antecedent speculation in this field, which had developed for a century before Plato's birth. They do not take account of all the relevant passages in the *Republic* itself, because of impatience to attribute some preconceived doctrine to Plato; they do not take much account of the eugenic programs in Plato's later dialogues, the *Statesman* and *Laws*, because without accurate knowledge of current theories and a complete picture of what the *Republic* says, the point of the later discussion is missed entirely.

In the present discussion, I intend to handle Platonic genetics as a part of the history of science; to present the relevant passages, and indicate what they mean in their historic context. Proceeding in this way, there are four key passages in the *Republic*, all of which represent possible social developments on the basis of the genetic theory presented in the *Statesman*. Depending on a society's success in understanding and applying it, this theory can lead to opposed historical consequences. So it seems at first glance that the *Republic* statements are mutually contradictory. Whether they are or not will be left for the reader to decide.

The first passage, *Republic* 424A, draws an optimistic conclusion concerning human progress from phenomena of animal-breeding. Paul Shorey (*Republic*, I, 331), translates:

> ". . . the state, if it once starts well, proceeds as it were in a cycle of growth. I mean that a sound nurture and education if kept up creates good natures in the state, and sound natures in turn receiving an education of this sort develop into better men than their predecessors both for other purposes and for the production of offspring as among animals also."

Republic 459A-B, to be quoted below, makes clear what is referred to by the final reference to "animals."

What this passage says is entirely compatible with the physiology of reproduction discussed by Plato in *Timaeus* 91A-D. "Seed" is formed from "marrow," and various illnesses and environmental changes will alter the qualities of this "seed-producing marrow." The marrow is the organ which coordinates the functioning of the whole organism and it is densest in the brain and the spinal column—an observation suggesting that Plato was familiar with accounts of back and head injury, and that his notion is less whimsical than his vocabulary makes it seem. As had apparently been suggested earlier by Empedocles, the whole organism contributes to the formation of spermata. But Plato's basic notions of physics would not accommodate Empedocles' further notion that one parental organism produced several kinds of "seed."

On the other hand, this passage gives only half the real picture; human im-

*The author has summarized, in three earlier discussions in the JOURNAL OF HEREDITY, some of the relevant stages in early Greek genetic speculation (the pre-Platonic theories of the Pythagorean school [from 529 B.C.], and of Empedocles [fl. 440 B.C.], and a development in the Hippocratic school roughly contemporary with Plato). I wish to thank Mr. Henry M. Butzel, Jr., of Indiana University for his expert advice on terminology and matrices, and Professor Tracy Sonneborn, Indiana University, for his explanations of current genetic theories. Their suggestions have been indispensable to me in my attempts to trace and present the development of genetic theory in the early Greek period, although responsibility for any errors remains my own.

provement would be inevitable if everything except education were held constant, and this improved; but there is no way of securing such constancy, and various factors tending to cause deterioration of the species are at work as well. Either the extent of improvement to be anticipated seemed not very great, or the probable balance of good and bad environmental factors seemed likely to persist, so that the present passage is not actually made the basis of deductions concerning human society and history in the subsequent discussion.[1]

In *Republic* 459, a second relevant passage appears. It has been agreed that in a just society, three classes of citizenry, specializing in the functions of legislation, protection, and production, should be established. Members of the first two groups will be chosen by competitive examination. The regulation of marriage is being discussed. Shorey (*Republic* I, 459-461) translates:

> "'I see that you have in your house hunting-dogs and a number of pedigreed cocks. Have you ever considered something about their unions and procreation?' 'What?', he said. 'In the first place,' I said, 'among these themselves, although they are a select breed, do not some prove better than the rest?' 'They do.' 'Do you then breed from all indiscriminantly, or are you careful to breed from the best?' 'From the best' . . . 'And if they are not thus bred, you expect, do you not, that your birds and hounds will greatly degenerate?' 'I do,' he said. 'And what of horses and other animals?', I said; 'is it otherwise with them?' 'It would be strange if it were,' said he. 'Gracious,' said I, 'dear friend, how imperative, then, is our need of the highest skill in our rulers, if the principle holds also for mankind.' 'Well, it does,' he said, 'but what of it?'"

The reader can no doubt see the answer to Glaucon's final question: among men, too, breeding must be as much as possible from the 'best' parent stock, or mankind "will greatly degenerate." To this end, marriages between the two upper classes and the artisan class will be prohibited, and arrangements will be made to have the best ruler and soldier pairs of parents produce children. Here we see the other aspect of experience with animal breeding: constant selection is required to prevent genetic deterioration.

However, there is to be equal opportunity for children, of whatever parentage, to compete for membership in the upper classes of the state; and, in *Republic* 415, Plato gives a "fiction" to be told the citizens in explaining genetics to them on a popular level. Suppose men's souls are of different metals, some gold, some silver, some iron and bronze. Still all are of the same species, and can breed together; while it is most likely that children will resemble their parents, still iron parents may have a golden child, gold an iron, and so on for all the combinations. Therefore we must have equal opportunity, for the most naturally talented citizens must be selected as soldiers and legislators. We also say that there is a prophecy that 'disaster will come to the state when it is ruled by a man of iron.'[2]

Many of Plato's readers have said this "fiction" is a downright lie, invented to placate the artisans. Plato, they argue, is an aristocrat and believes there never will be golden children born of iron parents; but he pretends this may happen, in order to make the artisans happy, while he really has set up an hereditary aristocracy. This contention is defended by assuming that Plato has in mind a combination-matrix showing the relation of talent between parents of each of the three class-levels and their children. The assumption of some latent matrix is almost certainly correct; the device is a frequent one in Plato's writings. The specific assumed matrix is certainly wrong, because it completely contradicts other sections of the text.[3]

Can we find any formula for parent-child relations of heredity that is: 1) a part of Plato's own knowledge, and 2) productive of the same results this "Myth of Metals" describes? If so, Plato's "popularized science" in an ideal state may be true except for the use of the simile of metals to give a simplified image of the seed-fusion producing different results. Of possible formulae, we can discard the Hippocratic and Empedoclean matrices based on discrete elements and non-homogeneous parental "seeds." These notions run counter to the "field" approaches about which Plato orients his entire scheme of natural science. We can also discard the notion that an average of parental endowment is inherited, and that this endowment is one of three grades, because that would make the birth of superior children to artisan parents, or inferior children to ruler parents, equally impossible. We can discard the more complex and plausible notion that an average is inherited, but since intellectual capacity and motivational tendency vary independently, though the soldier or ruler must excel in each, 81 combinations are theoretically possible.[4] It turns out that this matrix (which certainly fits the independent testing of temperament and intelligence—provided in the *Republic*—better than the 9-celled one first eliminated) has no theoretical provision for inferior children being born of superior parents, though it does predict that some marriages of artisans will produce superior children. The Pythagorean formula, suggested by taking literally the diagram of sides of a right-triangle determining a "resultant" hypotenuse,

which would make inherited capacity the square root of the sum of squares of parental capacities, though the diagram basic to it appears later in the *Republic*, must also be discarded. This calculation does provide for superior parents having inferior children, but not vice versa; and it further predicts an irreversible and alarming degeneration of human nature at a fantastic rate of speed, which history (in Plato's time, for example, the stability of government in Egypt) will not confirm.[5] In Plato's *Statesman*, it is suggested that in every evaluation, there is a norm with two possible directions of deviation, and that the good statesman uses normative measure in planning a eugenic program for the community.[6] The underlying idea of the "golden mean" is a traditional Greek doctrine. If, in applying it, we decide that our norm will be 0, and persons deviating by +1 or −1 fall in the ruling class, by +2 or −2 in the soldier class, by +3 or −3 in the artisan class, and that here (as in Plato's physics and astronomy) the resultant will equal the sum of component deviations, the 49-cell diagram of combinations fits the "Myth of Metals" exactly. Not only does it show the theoretical possibility of children of artisans being born rulers, children of soldiers being born artisans (and marriages between the ruler-soldier class must take place before final tests have separated the "gold" rulers from "silver" soldiers), it also shows the greater probability of superior children coming of superior parentage, thus fitting the earlier passage regarding animal breeding. Beyond this, the relative frequency of superior and degenerate types seems open to variation depending on social forces governing marriage; and, coinciding with some remarks in the *Statesman* about degeneracy, it predicts that repeated marriages of certain types will produce children further from the norm than either parent or anyone in the parents' generation.

It may seem that this elimination deliberately evades the point that restriction of marriage within class lines is not introduced into the *Republic* until some time *after* the Myth of Metals, so that the myth envisages all possible combinations of parentage, including those to be ruled out later. But, as we can see from examining the starred items in the diagrams under *Notes and References*, the assumption of all possible combinations *does not change the argument*: it still remains impossible for rulers to have bronze or iron children, or artisans to have gold ones, on just the assumption for which it was impossible with the class lines observed.

In other words, in considering that Plato's "fiction" may have some sound scientific foundation, which it presents in popular form, it appears that the precise theory of heredity used by Plato in a later dialogue: 1) predicts exactly the results the myth postulates, 2) justifies the expectation of greater relative frequency of talented children from talented parents, also postulated in the *Republic*; and 3) can be used to show how social betterment or decline will follow from the interaction of the basic laws of genetics with various social forces, leaving the way open for eugenic theories which like that cited earlier in the dialogue, predict human improvement, or, like the next passage to be cited, predict human decline. In addition, 4) the theory provides grounds for expecting the types of degeneracy of family line mentioned explicitly in the later political dialogues, though not in the *Republic*.[7] I think that these considerations should be conclusive as against the bizarre notion that equality of opportunity is a mere pretense to placate the masses, and the whole myth of metals is a cynical lie, contradicting the laws of genetics as Plato understood them.

In Table I, deviations of parents from the norm (= 0 deviation) are given as headings of rows and columns; predicted deviations of children of each pair of parents are given in the table. Note that any shift in social custom or law which changes the relative frequency of marriage between parents of any given pair of types will also change the average deviation of the next generation. This is, therefore, a *genetic* rather than a *eugenic* diagram.

The fourth key passage in the *Republic* is the "nuptial number," at 546A ff., explaining why even the best state would decline if it were placed in actual time and history.[8] Sooner or later, despite its rulers' knowledge of eugenics, the most talented rulers available would not be sufficiently well-endowed to see the good of the state clearly, and then national honor would dictate policy instead of national welfare. In a previous note, I have suggested that this passage is essentially Pythagorean. It is true, however, that the contingency it predicts would also be predictable from an extension of the Platonic figure given above. There would be a definite probability that at some point the best matches would not be fertile, or errors in planning would be made, with the result predicted by the Pythagorean "Muses" in the *Republic* 546 passage. The

TABLE I. Genetic matrix based on two directions and three degrees of deviation from a norm*

	+3	+2	+1	0	−1	−2	−3
+3	+6	+5	+4	+3	+2	+1	0
+2	+5	+4	+3	+2	+1	0	−1
+1	+4	+3	+2	+1	0	−1	−2
0	+3	+2	+1	0	−1	−2	−3
−1	+2	+1	0	−1	−2	−3	−4
−2	+1	0	−1	−2	−3	−4	−5
−3	0	−1	−2	−3	−4	−5	−6

*The ideal of character is here represented by 0 (no deviation); columns represent the deviation of one parent from this norm (as + or − 1, 2, or 3); rows represent the deviation of the other parent; the figures in the table predict the resultant deviations of their children. The interesting thing about this Platonic genetic combination table is that opposite extremes cancel out, so that one cannot predict the character of a child from knowledge of either parent alone.

earlier *Republic* passages cited above, make it clear however, that these Muses are rather over-pessimistic, and do not tell the whole story (though they are telling all of it explicable by the Pythagorean theory). The detail of this passage is not clear, and even the text is controversial; but the remarks made about it here are independent of such controversial points.

In conclusion, we may note that the two technical points of most interest in Plato's genetic speculation are first, his use of a scale with two directions of deviation instead of the Pythagorean scale which ranked the most desirable trait as one, and having only one direction of deviation (their rule of sum of squares would cancel out distinctions of positive and negative in any calculation). Second, Plato's awareness of the importance of knowledge of genetics for human social planning.

In the *Republic*, where a model state is described (as a standard by which to judge actual states, not as a practicable program), four different passages introduce four different aspects of Plato's building of eugenics on his basic genetic theory. Since children may be superior to their parents, equality of opportunity must be provided in our theoretical model of society; and the distance between this model and present social fact need not lead us to despair that men will never narrow the gap. Since children of superior parents are more likely to be superior than those of other parentage, the state would benefit from marriage laws precluding crossing of class lines (which are equivalent to lines between persons of different grades of talent)—if only one could identify the born ruler and born soldier accurately. In the *Republic*, the "ruler" and "soldier" simply personify social functions; needing direction and protection, Plato postulates citizens who are ideal directors and protectors. In an actual state, as the *Statesman* makes clear, people don't come in neat abstract categories, so the eugenic ideal can only be approximated. How this is done will be discussed later. Since children may be inferior to their parents, no actual society (however well ordered) can hope to avoid periods of adversity, in which first class leadership is not available. In the *Republic*, this probability of bad luck is made the start of a comparison of kinds of society through a hypothetical series of stages of decline of the model state. In an actual state, it appears that there are ways to provide for some stability in these stages of adversity; in the ideal model, since by hypothesis all power is centralized in the hands of the rulers, if we postulate one mistake on their part, the whole state is destroyed; but in concrete fact, states are less ideal and also much less brittle.

The suggestion Plato makes in setting up rules by which an actual community can improve is that there be provision for marriage-counselling based on scientific genetics. Young persons are to be told what sorts of marriage will be likely to produce the best endowed children, and it is hoped marriages of this type will become customary. As far as Plato's theory went, it seemed that marriages between persons of opposite temperament would be eugenically best, and this is the advice his marriage-counsellors would give. This seems to be a very sane and reasonable way to give practical import to the *Republic's* eugenic ideal, which rests on postulated "kinds" of persons that don't really exist.

The outcome of Plato's exploration of the theoretic and practical social importance of genetic theory shows Plato to have been a sensible and judicious explorer of a new field of inquiry, and far superior in these qualities to later propounders of strange interpretations of what they consider the eugenic and genetic ideas of Plato.

Notes and References

1. In the *Timaeus*, the effect of time is to produce progressive deterioration of the organism; perhaps this is the negative factor balancing possible environmental improvement. Had he not had the definition of justice as his main purpose, and the conviction that an ideal state must still have some degree of relevance to human nature as it is, Plato might have developed this notion of social improvement into a picture of a classless state, in which all citizens rotated duties of production, protection, and legislation.

2. *Republic* 415A-D. Shorey, *op cit.*, I, 305:

". . . While all of you in the city are brothers, we will say in our tale, yet God in fashioning those of you who are fitted to hold rule mingled gold in their generation, for which reason they are the most precious —but in the helpers silver, and iron and brass in the farmers and other craftsmen. And as you are all akin, though for the most part you will breed after your kinds, it may sometimes happen that a golden father would beget a silver son and that a golden offspring would come from a silver sire and that the rest would in like manner be born of one another . . . and if sons are born to them [the rulers] with an infusion of brass or iron, they . . . shall assign to each the status due to his nature . . . among the artisans and farmers. And again, if from these there is born a son with unexpected gold or silver in his composition they shall honor such and bid them go up higher, some to the office of guardian, some to the assistanceship, alleging that there is an oracle that the state shall then be overthrown when the man of iron or brass is its guardian. . . ."

3. Assuming no independence in inheritance of the various aptitudes a balanced excellence of which is sought in the ruler, the following

sort of matrix is envisaged:

	1	2	3
1	1	2	3*
2	2	2	3*
3	2*	3*	3

Here, 1, 2, and 3 represent the aptitudes of the three classes; in calculating heredity, the average is used, and the range 1.5–2 set = 2. Starred combinations are matches which cross class lines, and these will be prevented by marriage laws in the ideal society. If this figure were right, there would be no possible exceptions to the rule that the several classes would breed true. Nor could we explain the need for separate testing of character and intelligence that the text sets up. See note 4, below.

4. In this matrix, two figures are used to represent endowment; intelligence may be any one of three grades, and so may motivational predisposition. The result, putting intelligence first, of possible parental combinations is then the following:

	1–1	1–2	1–3	2–1	2–2	2–3	3–1	3–2	3–3
1–1	1–1	1–2	1–2*	2–1	2–1	2–2*	2–1*	2–1*	2–2*
1–2	1–2	1–2	1–3*	2–1	2–2	2–3*	2–1*	2–2*	2–3*
1–3	1–2*	2–3*	1–3	2–2*	2–3*	2–3	2–2+	2–3	2–3
2–1	2–1	2–1	2–2*	2–1	2–1	2–2*	3–1*	3–1*	3–2*
2–2	2–1	2–2	2–3*	2–1	2–2	2–3*	3–1*	3–2*	3–3*
2–3	2–2*	2–3*	2–3	2–2*	2–3*	2–3	3–2	3–3	3–3
3–1	2–1*	2–2*	2–2+	3–1*	3–2*	3–2	3–1	3–2	3–2
3–2	2–1*	2–2*	1–3	3–1*	3–2*	3–3	3–1	3–2	3–3
3–3	2–2*	2–3*	2–3	3–2*	3–3*	3–3	3–2	3–3	3–3

The row and column headings indicate parental talent.
* — indicates combinations resulting from marriages across class-lines, which are not to be permitted.
+ — indicates children of artisan parentage who qualify for admission to the upper classes (a score of 1 or 2 in each component is required to qualify).
The lowest level of inherited talent from parents both of the upper two levels is 2–2, the same as that of the most able children of artisan parentage.

5. The Pythagorean calculation of heredity would be:

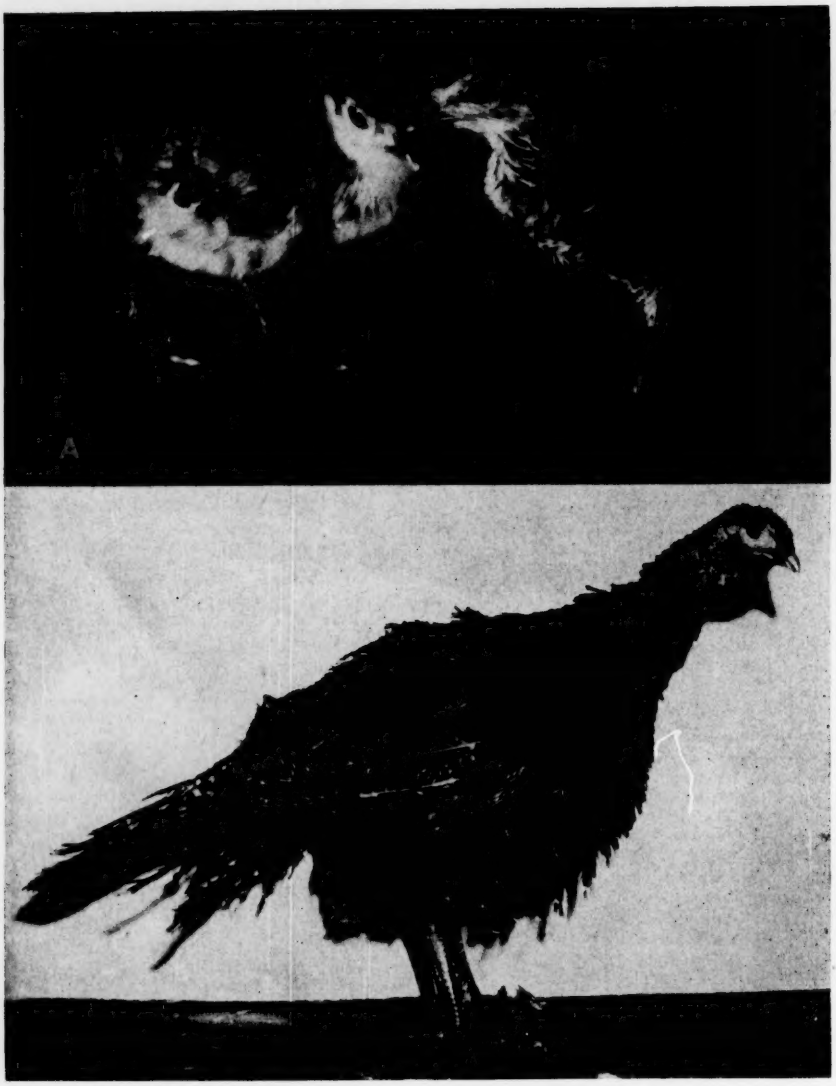
	1	2	3
1	$\sqrt{2}$	$\sqrt{5}$	$\sqrt{10}$*
2	$\sqrt{5}$	$\sqrt{8}$	$\sqrt{13}$*
3	$\sqrt{10}$*	$\sqrt{13}$*	$\sqrt{18}$

Used transitively, the next generation could at best have children of parentage $\sqrt{2}$ and $\sqrt{2}$, with a resulting highest endowment for these children of $\sqrt{8}$. As before, a star marks matings that would cross class lines.

6. *Statesman* 283C-285C. All useful arts "guard against exceeding the due measure or falling short of it . . . it is precisely by this effort they make to maintain the due measure that they achieve effectiveness and beauty in all that they produce" (*Plato's Statesman*, trans. by J. B. Skemp, p. 172). This is applied to the statesman's art of securing harmony in the state by "establishing intermarriages between the two types [the courageous temperaments and the controlled] . . . and by restricting private arrangements for marrying off daughters." *Statesman* 310B; Skemp, *op. cit.*, 232. The topic is discussed at length from *Statesman* 305E and 311D. These two passages (on measure and on its application to marriage) deserve careful reading in their context and their complete form.

7. The *Statesman* asserts that repeated strengthening of "spirit" or "irascibility" in a family line may end in mania; while strengthening of "docility" may end in imbecility.

8. *Republic* 546A ff. has occasioned more controversy as to its text and interpretation than any other passage in Plato. These various interpretations range from making Plato a fake geneticist, trying to impose on credulous readers, through making him an astrologer, to making the passage a kind of humorous aside. I have treated some details of the text and its interpretation elsewhere (*Plato's Mathematical Imagination*, Chap. 3, sec. 6 [Bloomington, Indiana, 1954]). For my present purpose, I only want to note that this passage in its context is only one of four related passages dealing with genetics and eugenics; that the other three are closely related to the known facts and current scientific theories of the time; and that the present statement reads like a recapitulation of the Pythagorean theory, which Plato adopts with crucial modifications in his later writings.



"HAIRY" POULT AND ADULT TURKEYS

Figure 20

A—One day old "hairy" (right) and normal poults. *B*—An adult "hairy" female, showing the general coarse, ragged plumage associated with this condition.

HAIRY, A GENE CAUSING ABNORMAL PLUMAGE IN THE TURKEY

J. Robert Smyth, Jr.*

During the Summer of 1950 the attention of the author was called to the presence, on a Massachusetts farm, of several Broad-breasted Bronze turkeys with an extremely abnormal type of plumage. That fall one male and three females were obtained for study. Subsequently, the same mutation was found in the flocks of two other turkey breeders in the same area. All three had recently purchased Broadbreasted Bronze stock from the same Pacific Coast breeder. This type of abnormal plumage had been generally referred to by turkey growers as "hairy" for several years before the author was aware such a condition existed. Normal feather webbing was absent in the adult mutant. Instead, the individual feathers appeared to have short thick hairs attached to the rachis. Since the down plumules were also extremely modified and had the appearance of coarse hair, it is suggested that the original name "hairy" be retained for this feather trait.

Description

Adults—Adult hairy turkeys are characterized by abnormal feather structure in all pterylae. As illustrated in Figure 20*B*, the general appearance is one of a turkey with coarse hairy or ragged plumage. A microscopic examination of individual hairy feathers showed an absence of normal webbing (Figure 21). Each barb was completely separated from those on either side of it resulting in a treelike appearance. With the exception of the feather abnormality, hairy turkeys appeared to be normal.

A microscopic examination of the modified web region of hairy breast and back feathers showed the barbules to be greatly reduced in number and to be of abnormal structure. Except for the presence of occasional groups of small, poorly formed distal barbules, the barbules were absent except on the base portion of the barb. This barbulated base portion usually included the proximal one-tenth to one-fifth of the barb. Within this base region where barbules were common, the distal barbules were quite numerous, but the proximals were less evident except very near the region of the barb's attachment to the rachis. The distal barbules were shorter than those of normal feathers, but appeared to have normal hamuli or barbicels. The structure of the fluff or normally non-webbed portion of the hairy feather showed no marked formational differences when compared with normal feather structure. However, the individual barbules did tend to lie closer to the barbs, resulting in a less fluffy appearance than is evident in the non-webbed portion of the normal feather.

From casual observation, the primary and secondary wing feathers appeared to be narrower in the web region (Figure 21). Closer inspection showed that the barbs are of normal length but lie at an approximate angle of 25° from the rachis in comparison with the normal 60 to 70° angle. In the secondaries the barbs were curled towards the underside of the rachis, giving the impression of a cylinder. The proximal barbules were completely missing in most cases. The distals were fewer than normal in number and greatly reduced in size, although as in the hairy body feathers hamuli were present.

The main tail feathers of the adult hairy were found to be similar in structure to the body feathers. However, the tail feathers showed fewer proximal barbules, had smaller distal barbules and fewer hamuli than were observed in the back and breast feathers.

*Massachusetts Agricultural Experiment Station, Amherst. Contribution Number 924.

Poults—The down of newly hatched hairy poults appeared wiry or like coarse hairs (Figure 20*A*). The affected down lay closer to the skin than normal down. The skin on the back of the poult was frequently visible, as if the down had been unevenly pasted to the back. Hairy poults were detected with complete accuracy at hatching time. In fact it was possible to remove poults that were alive but unable to escape from the egg or that had died after 25 days of development, wash them thoroughly in water, dry them off, and classify them as hairy or normal.

The down was abnormal in all pterylae, and, with the exception of the more nearly normal head down, all areas appeared to be affected similarly.

Under a microscope the hairy down showed varying modifications from normal down. The most extreme modification showed little or no branching of the barbs from the short down quill as is found in normal down. In this type of down the barbs appeared to be combined into one shaft with only an occasional branching. In the type of down showing the least modification some normal branching of the barbs was present. Barbules, although fewer in number than in normal down, were found on these barbs. Down plumules intermediate to these two extremes were more common than the above. The down on the head was affected the least in the hairy poults but showed some effect in that many of the barbules cohered and in a few head plumules the number of barbules was greatly reduced.

Genetics

Since the hairy condition resulted from matings of normal by normal in the turkey flock that provided the hairy stock used in this study, the original assumption was that it was recessive in nature. As can be seen in Table I, subsequent matings showed the hairy condition to be due to a single recessive gene. That the hairy gene is autosomal was established by the presence of only normal males and normal females among the offspring of the hairy male by normal female mating. A satisfactory gene symbol for the hairy condition would appear to be *ha*, which to the author's knowledge has not been assigned to an inherited character in the chicken or the turkey.

The turkeys used in these crosses to represent normal feathering were genetically pure for the red plumage color of the Bourbon Red variety. The red feather coloration differs from the bronze color pattern by a single recessive autosomal gene *r*.[7]

The expected ratio for independent segregation in the F_2 mating was 9 normal bronze (*Ha-R*—) : 3 normal reds (*Ha-rr*) : 3 hairy bronze (*haha R*—) : 1 hairy red (*haharr*). The observed ratio did not differ significantly from the expected ($\chi^2 = 5.646$; $p > .10$). It should be noted that the only marked deviation from the expected occurred in the hairy red class. Such a deviation might indicate linkage between the non-red (*R*) and hairy (*ha*) genes or some interallelic relationship between these two genes. To obtain more information on this possibility the three degrees of freedom were separated into three separate components, in which one degree of freedom was used to test for discrepancies in the *Rr* factor pair segregation, one to test the *Haha* factor pair segregation, and the third degree of freedom to test

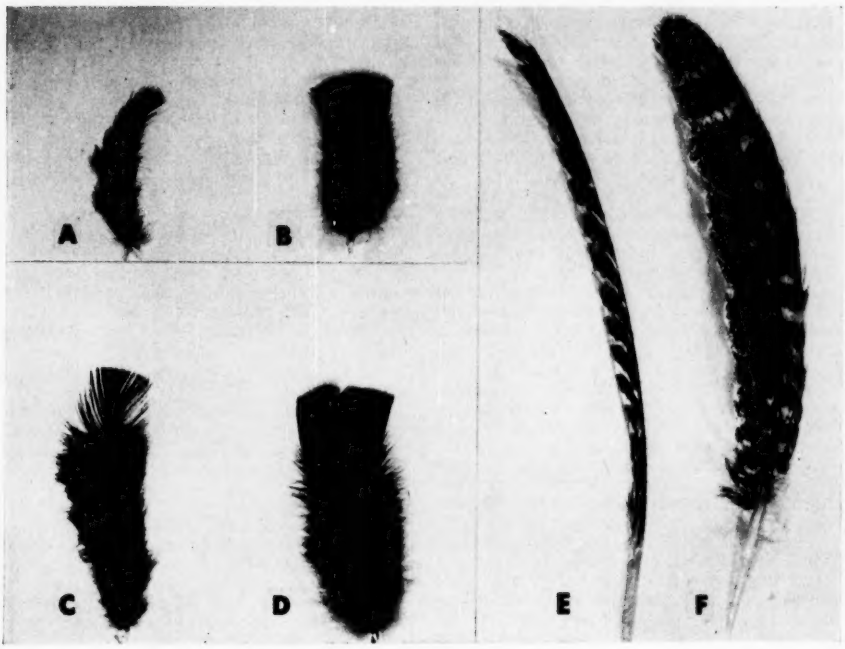
TABLE II. Hatchability in matings concerned with the hairy condition

Type of Mating	Total Fertile Eggs	Percent Embryos Dying 0-24 Days	Percent Embryos Dying 25-29 Days	Percent Hatched
Hairy ♂ × Hairy ♀	53	9.4	28.3	62.3
Backcross (Hairy ♂ × F_1 ♀)	53	7.6	7.6	84.8
F_2 (F_1 ♂ × F_1 ♀)	84	7.1	16.7	76.2
F_2 (Normal stock)*	625	6.5	16.1	77.4

*An F_2 from the same varietal cross but utilizing stock not carrying the hairy gene.

TABLE I. Results of matings involving the hairy condition

Mating Type	Normal bronze	Normal Red	Hairy Bronze	Hairy Red	χ^2	P
Hairy bronze ♂ × Hairy bronze ♀	0	0	55	0		
Normal red ♂ × Hairy bronze ♀	{ 3♀ 4♂	0	0	0		
Hairy bronze ♂ × Normal red ♀	{12♀ 10♂	0	0	0		
Hairy bronze ♂ × F_1 ♀	31	0	34	0	0.138	> .70
F_2	62	24	21	1	5.646	> .10



"HAIRY" AND NORMAL FEATHERS

Figure 21

Adult feathers showing differences between the "hairy" and normal condition. *A*—Hairy breast feather. *B*—Normal breast feather. *C*—Hairy back feather. *D*—Normal back feather. *E*—Hairy wing secondary. *F*—Normal wing secondary.

for iinkage between the two. The results of this test are as follows:

Non-red: red segregation component,
$\chi^2 = 1.23$ ($P > .25$)
Normal: hairy segregation component,
$\chi^2 = 1.00$ ($P > .30$)
Linkage component,
$\chi^2 = 4.21$ ($P < .05$)

Therefore, although the χ^2 for goodness of fit for the 9:3:3:1 ratio was not a statistically significant deviation from the expected, the χ^2 test for linkage indicated possible linkage between the two factor pairs. Additional matings will be necessary before a linkage relationship can be definitely established.

Viability of Hairys

When reared in competition with normal poults the mortality of hairys was higher than in those with normal plumage. Out of a total of 25 hairy poults started, 20 percent or five died during the first 12 weeks of life. Under identical conditions only 6.4 percent of the normal 565 poults died. However, it should be pointed out that most of the hairys died from cannibalism. Such habits are difficult to control in normal turkeys, and the abnormal appearance of the hairys marked them as a choice target. Similar results have been observed at this station when small numbers of poults with white plumage were reared with large numbers of turkeys with colored plumage. Although the author has no data on viability of range-reared hairys, breeders report that hairys survive and develop satisfactorily if climatic conditions are good, but suffer heavier than normal losses following heavy rains. This is no doubt a reflection of poorer protection afforded by their abnormal plumage. In a sense the hairy can be thought of as a partial facultative lethal under the usual commercial conditions.

In order to ascertain whether embryonic mortality is greater in hairys than in normal turkeys the available hatchability data are incorporated in Table II. Although the numbers involved were admittedly small, it appeared that some information could be ob-

tained on this point. The backcross of F_1 females to a hairy male resulted in a high hatchability of 84.9 percent indicating that the hairy male contributed no deleterious effects to embryonic development. The extremely low hatchability of the hairy hens in the hairy by hairy mating suggested that hairy females fail to provide as desirable an environment for development within their eggs as do birds with normal feathering. A similar hatchability problem has been reported in the case of the Frizzle fowl by Landauer and Dunn.[5] In reviewing this problem in Frizzle fowl Landauer[6] indicated that the excessive loss of body heat through the defective feathers characteristic of the Frizzles leads to disturbances in temperature regulation and metabolism which results in an increased embryo mortality rate. Although the hairy by hairy cross is the only one included here which did not involve a varietal cross, it seems unlikely that the absence of heterosis could explain the resulting low hatchability from this mating. Also the breeder from which the stock was obtained reported that the hatchability in his pure Bronze matings was approximately 80 percent.

These data would also appear to shed some light on whether hairy poults from normal dams are less apt to hatch than are their normal sibs. Hatchability data from an F_2 cross involving the same strains, but not carrying *ha* was 77.4 percent which was similar to the 76.2 percent hatch for the F_2 hairy cross and was less than the 84.8 percent hatch for the backcross. Since the hatchability of the backcross from which one-half the poults were hairy is higher than in the F_2 cross from which no hairys were involved, it would appear that the hairy condition itself has no deleterious effect on an individual poult's chances of successfully emerging from the egg.

Discussion

Genes which control the developmental processes involved in feather formation in the domestic fowl have provided an excellent source of genetic material for study. Hutt[2] has recently reviewed 16 specific gene mutations affecting in different ways the plumage of the chicken. Of these, silky, frizzling, flightless, fray, and naked act similarly to the hairy gene in that they cause defective feather structure within the normal pterylae. In addition, three more such genes have been described. These include ropy,[8] sunsuit[3] or stringy[1] and a separate sublethal mutation affecting the down also referred to as "stringy".[4] On the other hand, to the author's knowledge, hairy is the first reported mutation affecting structure of the plumage in the turkey. A review of the descriptions of the feather mutations in the chicken indicates that the hairy condition in turkeys represents a type that is different from any previously reported.

In one of the Bronze flocks that showed the hairy condition the incidence was reported to be two percent of all poults hatched. In this population the gene frequency for the hairy gene was .142. Since hairys are classed as cull poults, they have no sale value to the commercial poult producer. Although most producers attempt to raise them as meat birds, the greater than average mortality of the hairys in competition with normal stock results in a higher cost per pound of turkey meat produced by the mutants. The elimination of the hairy gene from commercial strains of turkeys would be highly desirable.

Summary

A description has been given of an autosomal, recessive mutation in the turkey resulting in abnormal down and plumage. Normal feather webbing was absent in the adult mutant. Instead, the individual feathers appeared to have short thick hairs attached to the rachis. Since the down plumules were also extremely modified and gave the appearance of coarse hair, the name "hairy" is suggested for this condition. The "hairy" gene appeared to have no deleterious effects on embryonic development and hatchability, but under commercial growing conditions "hairy" turkeys showed a reduced viability.

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CHESTNUT BREEDING, TECHNIQUES AND RESULTS

II. Inheritance of Characters, Breeding for Vigor, and Mutations

RUSSELL B. CLAPPER

Blight Resistance

GRAVES[6,7] and the writer[3] determined the relative resistance of different species and hybrids of *Castanea* to the blight fungus (*Endothia parasitica*). Both investigators agreed that blight resistance of first-generation hybrids from *C. mollissima* and *C. dentata* is intermediate between the resistance of *C. mollissima* and the susceptibility of *C. dentata*. The writer made large numbers of backcrosses of *C. mollissima* × *C. dentata* hybrids to *C. mollissima*, and the backcross-generation trees have shown, both from natural and artificial infections, a resistance to blight nearly equal to that of the *C. mollissima* parent trees. This fact suggests that more than one pair of genes controls the inheritance of blight-resistance. Furthermore, a frequency distribution of the areas of cankers in the backcross-generation trees indicates that two pairs of genes control the inheritance of blight resistance.[3] Additional investigation of the inheritance of blight resistance is needed, especially of second-generation hybrids from resistant × susceptible crosses.

Early Flowering

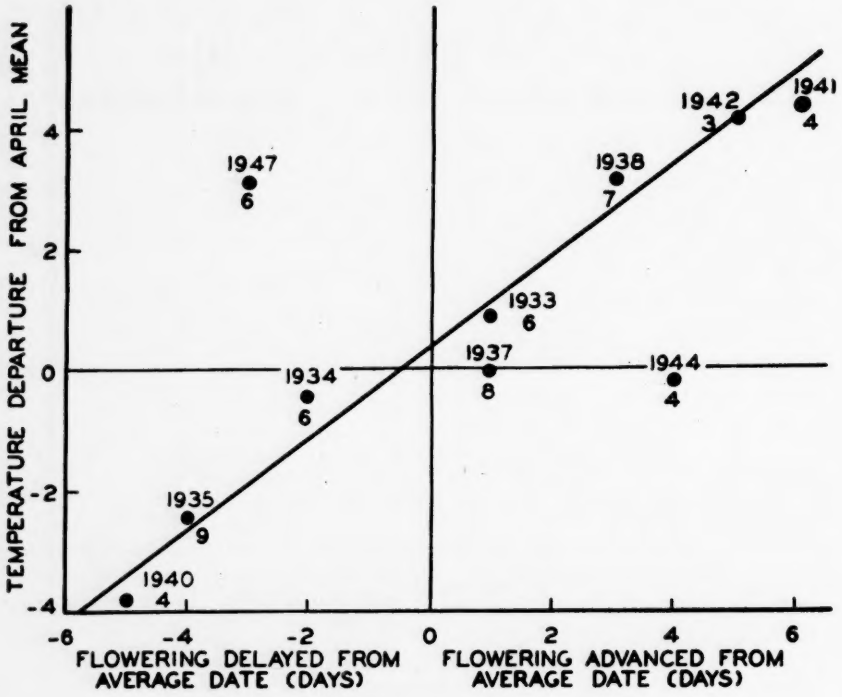
In this study, the beginning of flowering in chestnut and chinkapin was considered to be the date on which the first several staminate catkins began to blossom. Over a number of years there are usually a few in which flowering occurs unusually early or unusually late, due to abnormal climatic variations in the spring. When compiling data on the inheritance of early flowering, the extreme dates were omitted. The residual variation in flowering dates of a given species or hybrid from year to year may then be accounted for by normal climatic variations, and an average of such flowering dates will more nearly represent the basic flowering date of the tree or trees under study.

Records of dates of flowering for one to nine years, of 16 parent trees and 57 F_1 hybrids, comprising seven different interspecific crosses, show that the hybrids flower at least as early as the earlier parent. Dominance of early flowering was also found in 66 hybrids obtained by crossing three species with different hybrids; in 51 progenies from intercrosses and inbreds, and in 195 backcross-generation trees from seven different backcrosses. In segregating generations, as the second and backcross generations, earliness sometimes exceeded that of the earlier parent, probably due to transgressive segregation.

Early flowering of chestnut and chinkapin species and hybrids results in earlier ripening of the nuts, and this is important if chestnut weevils are present in the planting.

The effect of departure of spring temperatures from the mean on the deviation of flowering time from the average was investigated. Temperature data for Glenn Dale, Md., were obtained from the Climatological Data of the U. S. Weather Bureau. These data were obtained for 10 years; in four of these years flowering occurred earlier than the average dates, in two flowering occurred about the average time, and in four others flowering was later than the average dates. Temperature departures from the means were obtaind for the months of March, April, May and June for each of the 10 years.

The average flowering dates of 11 trees, and the deviations from these dates were obtained for each of the 10 years. The trees included three Van Fleet hybrids, two of *C. mollissima*, two



EFFECTS OF TEMPERATURE ON FLOWERING

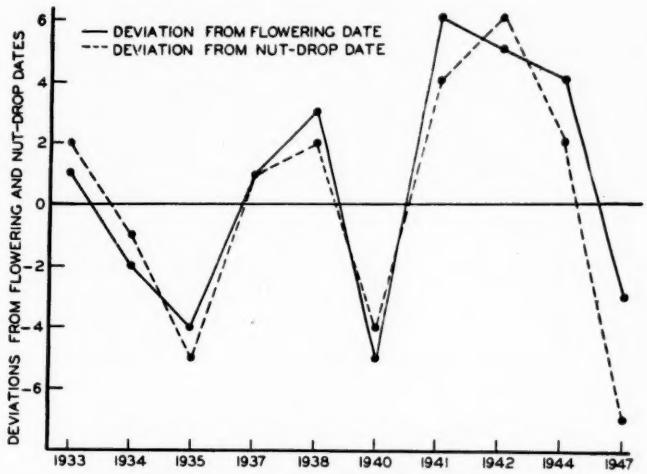
Figure 22

Effect of temperature departures from the April mean on flowering time of chestnut species and hybrids at Glenn Dale, Maryland.

of *C. crenata*, and one each of *C. pumila*, *C. henryi*, *C. dentata*, and *C. seguinii*. The average flowering times of these trees ranged from very early for *C. henryi*, early for the three hybrids, one *C. mollissima*, one *C. crenata*, and *C. pumila*, medium late for one *C. mollissima*, one *C. crenata*, and *C. seguinii*, to very late for *C. dentata*. At Glenn Dale, chestnut buds begin to swell and leaf out in April, and flowering for most species and hybrids begins between June 1 and June 20.

Temperature departures from the mean were plotted with the average deviations from the average flowering date of the several trees for the ten years. The departures from the mean for April of the ten years (Figure 22) showed a greater influence on flowering time than those for any other month or combination of months.

The points on the graph (Figure 22) for 1944 and 1947 are widely dispersed from the general distribution. The average temperature for April 1944 was nearly normal (departure of —0.1°); however, the high average for May (departure of 6.2°) advanced the flowering time four days in 1944. In 1947, flowering occurred three days later than the average date, although April was very warm (departure of 3.2°). May was near normal (departure of 0.5°), but the low average for June (departure of —2.4°) delayed the flowering time. Any



FLOWERING AND NUT-DROP DEVIATIONS

Figure 23

Effect of deviation from average flowering date on deviation from average nut-drop date.

species or hybrid at Glenn Dale that blossoms in May, as *C. henryi* does, will not of course be affected by a cold June.

When average temperature for both May and June is nearly normal, the April temperature departure is the most significant for predicting whether flowering will occur early, at the average time, or late. If the average temperature for April is low and that for May is high, or if April is high and May is low, the respective departures from the means are added and divided by two to obtain the average departure for two months. Predictions made on the basis of April or April and May temperature departures will be affected by June temperatures only in case the latter are abnormally low. March temperature departures have no effect on chestnut flowering time at Glenn Dale, Md., since bud growth usually begins in April. Also, departures from the mean of precipitation for the spring months have no significant effect on flowering time.

Time of Nut Drop

In some years nut drop begins early or late. The exceptional dates were eliminated in determining the average date of nut drop. Records of 35 F_1 hybrids for one to six years from three different interspecific crosses show that time of nut drop is inherited without dominance, that is, as an intermediate character. Records of 35 trees from three different backcrosses for one to four years show that time of nut drop favors that of the earlier backcross parent. Nut drop earlier than the earlier parent is rare in individual trees of the backcross generation or of the first generation.

An investigation was made of the climatological factors that may affect the time of nut drop. Temperature and precipitation departures from the means for July, August, and September were obtained for 10 years. Also, the average deviations from the average date of nut drop were obtained for 14 trees including two of *C. crenata*, seven of *C. mollissima*, and five hybrids, for each of the 10 years. Mean minimum temperatures, and the percentage of sunshine for the same months and years were also obtained. Analyses of these factors showed that they had little effect on time of nut drop.

Deviations from average date of flowering were then compared with deviations from average date of nut drop, and these were found to coincide closely (Figure 23). Insofar, then, as April temperatures determine the time of flowering (at Glenn Dale, Md.), they also determine the time of nut drop. These observations lead to the conclusion that the reproductive phase (as defined later) of a species, race, or hybrid of *Castanea* tends to be a fixed number of days, just as the analogous gestation

period of animals tends to be a fixed number of days.

Length of Reproductive Phase

In this study, length of reproductive phase is the number of days from the beginning date of flowering to the beginning date of nut drop. The preceding sections show that earliness of flowering in *Castanea* is dominant to lateness, and time of nut drop is intermediate. The progenies of crosses then, with some exceptions, will tend to have longer reproductive phases than either parent. This was found to be the case with most hybrids from interspecific and intervarietal crosses. For example, two early flowering and early dropping hybrids, 016 and S8, have reproductive phases of 88 days. Twenty-one offspring from crossing these hybrids had a reproductive phase ranging from 92 to 109 days. A large number of offspring no doubt would have extended the range in both directions.

At Glenn Dale, Md., the reproductive phases of several introductions of *C. mollissima* ranged from 89 to 107 days; *C. crenata* ranged from 93 to 109 days, that of *C. pumila* was 97, *C. dentata* 115, *C. ozarkensis* 116, and *C. henryi* 143 days.

Nut Size and Number of Nuts to the Burr

The size of nuts from a given tree will vary from year to year, and in certain years the size may be unusually large or unusually small. The abnormal nut size was eliminated when determining average nut size. Records for one to 10 years on 80 F_1 hybrids from five different interspecific crosses show that nut size is inherited as an intermediate character, but tends to approach the nut size of the parent producing the smaller nuts. Records from one to seven years on 225 trees from five different backcrosses made to the small-nut parent show that nut size tends to approach or equal that of the small-nut parent tree. Thus, small nut size appears to be partially dominant over large nut size. Also, the average weight of hybrid nuts is nearer the geometric mean than the arithmetic mean of the parental nut weights. Second-generation trees from interspecific crosses were insufficient in number or too young to yield additional data on the inheritance of nut size. Detlefsen and Ruth,[5] however, noted that the F_2 nuts from a *C. crenata* × *C. dentata* cross made by Endicott ranged in size from that of *C. dentata* to that of *C. crenata*, indicating that nut size is a quantitative character, the inheritance of which is controlled by multiple genes.

Size of nuts from a given tree varies from year to year, due to changing environmental factors, including the amount of precipitation preceding nut drop, competition for soil moisture, and soil fertility. Analyses of the precipitation departures from the mean for July and August, and the deviations from average nut weights for two to 15 trees, for 10 years at Glenn Dale, Md., showed that the weights of the nuts increased with increased precipitation, and decreased when precipitation decreased during these months.

Insufficient cross pollination will reduce the number of chestnuts in a burr from three to two or one, and these usually will be larger. McKay and Crane[9] and the writer's unpublished data show that the size of nuts produced by a tree may be increased or decreased by using pollen from another tree that bears larger or smaller nuts. This effect is due to the immediate effect of the pollen on the development of the embryo.

A large crop of nuts following a small crop, or a small crop following a large crop, usually affects the size of the nuts. Records of 61 hybrids three to eight years old gave 105 comparisons, 79 percent of which showed that when the burr crop increased or decreased when compared with the previous year's crop, the average nut weight decreased or increased. The remaining 21 percent of the cases did not bear out this inverse relationship.

Records taken for eight to 16 years on nine seedling trees and topgrafts indicate that, for trees up to 35 years old, aging does not affect nut size.

By definition, chestnuts have two or more, usually three, nuts to the burr, and chinkapins have one to the burr. When a chestnut and chinkapin are crossed, the first-generation trees usually bear one nut to the burr. Second-generation trees from such a cross were insufficient in number to determine how the single-nut character is inherited.

Color of Nutshells

Nutshells of the various chinkapins in this country are near-black to black in color. Shells of the Henry chinkapin of China are chestnut brown in color, as are most varieties of the Japanese chestnuts. Shells of various Chinese chestnuts range from a muddy brown color to bright purplish-black; a few varieties produce chestnut-colored nuts. First-generation trees from crossing native chinkapins with chestnut-colored chestnut species or with Henry chinkapin yield nuts that are reddish-black to black in color. Nuts of first-generation trees of *C. mollissima* × *C. crenata* are usually of a darker chestnut color than that of nuts of *C. crenata*. Shell color in nuts of *Castanea* appears to be a quantitative character that is controlled by a number of genes.

Nut Flavor

The flavor of American chestnuts, distinctly sweet and aromatic, probably surpasses that of all other chestnuts. The Oriental chestnuts lack the aroma but some selections of the Chinese chestnut make up for it in sweetness. Sweetness and aroma are inherited in an intermediate degree in nuts of first-generation

HYBRID CHESTNUT TREES
Figure 24

A group of first generation hybrids from crossing *C. mollissima* M16 with *C. dentata* is shown on the left. Superficial blight cankers on trunks of the F_1 trees increase their apparent taper. On the right is shown a 9-year-old graft of Chinese chestnut on Japanese chestnut stock. The scion was topworked at the point indicated.

trees. This fact makes it difficult to breed a blight-resistant chestnut with sweet and aromatic nuts; to obtain these qualities, one must backcross to the American chestnut but this increases the blight susceptibility of the hybrids.

Everbearing

"Everbearing" means the successive development of involucres along the branch as the latter extends its growth. The burrs at the lower end of the branch mature first, the succession of maturing burrs proceeding toward the terminal of the branch. The character appears in most introductions of the Seguin chestnut and is also found in forms of the Japanese chestnut growing in southern Japan. A variation of everbearing is the two- or three-crop habit, where two or three distinct crops of burrs mature in one season. Everbearing is a dominant character in first-generation hybrids from crossing non-everbearing with everbearing, and is inherited in a ratio of at least three to one in the second generation, with the character varying from the two-crop type to true everbearing.

Breeding Chestnuts for Vigor

Chestnut trees must be cross-fertilized to set viable seed. In the forest this means that those chestnut trees in close proximity will intercross more effectively than trees more widely separated. Sexual reproduction of the chestnut in the forest thus involves such methods of loose inbreeding as parent-offspring, brother-sister, and first-cousin matings. The American chestnut has reproduced by these forms of inbreeding for hundreds of generations, yet this forest tree had remarkable vigor. Its vigor may be explained genetically by assuming that

the species carried one or more major genes for vigorous growth, and the various forms of natural inbreeding maintained these genes in a homozygous condition, so that succeeding offspring always carried these dominant genes. Other dominant, homozygous genes similarly controlled other characters in the chestnut, so that from generation to generation it reproduced with little discernible change. Jones[8] stated, "The long and vigorous selection which wild species had to undergo under natural conditions makes it possible that cross-fertilized plants in the open may be in a state of near homozygosity, even though continually crossed. Many of them are as uniform and fixed in their type as self-fertilized species under the same conditions . . ."

An alternative theory to explain the vigor of loosely inbred forest species such as chestnut was advanced by Brieger,[2] who cited facts to show that the hypothesis of dominant genes does not provide a satisfactory explanation of all the results obtained by inbreeding. He believed that the theory of heterotic gene interaction forms an adequate basis for interpreting the results of inbreeding. He further supported his theory as follows: inbred lines possess residual heterozygosity, the existence of balanced heterotic populations in indigenous South American maize, and the possibility of obtaining such balanced populations by breeding.

Chestnut breeders strive to obtain blight-resistant hybrids that are at least as vigorous as the American chestnut. The task has not been an easy one. The species of Asiatic chestnuts are smaller in stature than the American chestnut, and hybrid vigor, which sometimes occurs in the first-generation from interspecific crosses in other genera, is not comomn in such crosses in the genus *Castanea*.

Several types of Chinese chestnuts growing at Glenn Dale, Md., were crossed with a few American chestnut sprouts. Some of the first-generation progenies showed considerable susceptibility to blight and poor tree form. The progeny of one cross (*C. mollissima* × *C. dentata*), however, appears to be superior in blight resistance, tree form, and growth rate. The form of these trees is similar to that of the American chestnut (Figure 24), while the size, color, flavor, and downiness of the nuts, and the density and length of the spines are intermediate between these characters of the parent trees.

It was not practical at Glenn Dale, Md., to obtain a direct comparison of the growth rate of these superior first-generation hybrids with that of the American chestnut because of the latter's susceptibility to the blight. It is not known if these trees have hybrid vigor in the sense that the hybrids are more vigorous than either parent; however, in the sense of the term as used by Brieger,[2] the hybrids are more vigorous than their progeny obtained by any form of inbreeding. They were backcrossed to the same *C. mollissima* parent tree, and the backcross generation averaged 2.1 feet of growth per year for the first nine years, compared with 2.8 feet for the F_1 hybrids for the first 10 years. The F_1 trees were also backcrossed to other types of *C. mollissima* in an attempt to introduce genetic diversity in the backcross generation. This attempt did not produce superior growth rates over that already obtained. The F_1 trees were also backcrossed to American chestnut in recent years, but the progeny is too young to make fair comparisons of growth rate and blight resistance.

A rate of growth of two feet per year is considered fair for chestnut species and hybrids growing on the poor site at Glenn Dale. This is about the maximum rate of growth obtained with Chinese chestnuts on selected forest sites.[4] First-generation hybrids of certain interspecific crosses between the tree chestnuts have grown at a rate greater than two feet per year at Glenn Dale. Less vigorous growth was made by progenies from crosses within species, by the backcross generations of *C. mollissima* × *C. dentata* backcrossed on *C. mollissima*, and by progenies obtained by inbreeding the Van Fleet hybrid 016 (Table I).

The blight-resistant, fast-growing *C. mollissima* × *C. crenata* hybrids probably would make good timber trees in forest plantings; however, *C. crenata* is somewhat inferior to *C. mollissima* in resistance to blight and drought, and decidely so in the quality of its nuts. Within recent years, numerous crosses have been made between different types of

TABLE I. Comparison of growth rates of interspecific and other types of chestnut crosses at Glenn Dale, Maryland.

Number of trees	Cross	Average growth rate per year (feet)	Age of trees (years)
4	*C. mollissima* T4 × *C. crenata* FP. 58	2.5	10
5	*C. crenata*-w* GF × *C. mollissima* FP. 56	2.5	8
5	*C. crenata*-w GF × *C. dentata* FP. 555	2.2	10
51	*C. mollissima* M16 × *C. dentata* FP. 555	2.8	10
4	*C. mollissima* M16 × (M16 × *C. dentata* FP. 551)	2.1	9
10	*C. crenata* FP. 48 × (*C. mollissima* M16 × *C. dentata* FP. 551)	1.9	5
19	*C. crenata* FP. 45 × *C. crenata* FP. 48	1.3	11
14	*C. mollissima* FP. 801C × *C. mollissima* FP. 821	2.0	9
16	(*C. mollissima* × *C. crenata*) 05 × (*C. mollissima* × *C. crenata*) M6	2.4	8
10	016 × 016 F_2†	1.8	6
15	016 × (016 × 016) F_2	1.4	8
2	(016 × 016) F_2 × (016 × 016) F_2	0.8	5

* The wild form of *C. crenata*.

† 016 is a *C. mollissima* × *C. crenata* hybrid that sets some seed by its own pollen.

C. mollissima in an attempt to obtain faster growth rate. Also, different types of *C. mollissima* have been crossed with *C. dentata* within the last 10 years in an attempt to obtain still more blight-resistant and faster growing first-generation trees for forest plantings.

Inbred lines of corn, when combined, form hybrids that have a high degree of vigor. If forest trees could be bred similarly, races of superior trees might be obtained. However, some forest-tree species, including chestnut, seldom set seed by their own pollen so that the operation of selfing, usually necessary for close inbreeding, is not available. Brother-sister mating is less rapid in attaining homozygosity than selfing, and may lead to no greater homozygosity than is found in the species in nature.

Since the Van Fleet Chinese chestnut hybrid 016 is partly self-fertile, a number of second-generation seedlings obtained by selfing 016 were obtained. These were also selfed in an attempt to find a tree with greater self-fertility than the parent hybrid. Only four of nine F_2 seedlings set nuts by their own pollen; the highest set for a single tree was 9.8 percent. A set of 8.6 percent was obtained by selfing the parent hybrid. Growth rates of 016 progenies obtained by selfing and other methods of inbreeding diminished rapidly as inbreeding advanced (Table I).

Mutations in *Castanea*

In 1928 R. Kent Beattie of the Division of Forest Pathology shipped to this country scions from a Japanese chestnut tree that bore spineless burrs. In 1940, grafts of spineless were crossed with *C. mollissima, C. crenata, C. henryi,* and *C. pumila.* Some of the hybrids fruited but the burrs were not spineless. The mutation no doubt is recessive and may appear in second generations or in backcross generations where the backcross is made to the mutant parent tree.

The mutation called "cracked bark" occurred in first-generation hybrids of *C. mollissima* M11 × *C. dentata,* a cross made in 1935. In the same year *C. mollissima* M16 was also crossed with the same specimen of *C. dentata.* There were no visible differences between the parent trees M11 and M16 except that branches of the former made slightly wider angles with the stem. However, hybrids of M11 × *C. dentata* displayed cracked bark in the third year of growth and drooping to upright branches later. The proportion of bark to wood in the mutant trees was greater than normal. Trunk bark of *C. mollissima* × *C. dentata* hybrids normally does not crack until the trees are 15 or more years old.

Comparisons of various characteristics of the cracked-bark and the normal hybrids were made when they were six years old. Sixty-two percent of the 60 cracked-bark trees had blight cankers, and 46 percent of the 41 normal trees had blight cankers. Forty percent of the cracked-bark trees were affected with dieback (winterkilling), whereas none of the normal trees was affected. The average height of cracked-bark trees at six years was 9.1 feet, of the normal trees 15.5 feet.

In 1942 the parent trees M11 and M16 were intercrossed reciprocally to determine if cracked bark would appear in the first-generation trees. A total of 74 seedlings were obtained, and these were observed for six years but the mutation did not reappear.

Another anomoly, "naked chestnuts," is probably a nonheritable mutation because it occurs only in certain years on affected trees. The burrs stop growing before they develop fully, while the nuts develop normally. As a result, the burr breaks apart between its base and apex, or breaks open at the apex, and the green nuts are uncovered.

Vegetative Propagation of *Castanea*

Chestnuts and chinkapins graft readily, when stock and scion are dormant and a perfect fitting of stock and scion is obtained. Interspecific grafts, in the form of bench grafts or topworked grafts have been made successfully. One of the latter, a Chinese chestnut topworked in 1928 on a 15-year-old Japanese chestnut, is now 25 years old and produces crops of nuts yearly (Figure 24).

The budding of chestnut apparently is not as successful in the eastern States as it is on the West Coast. Tests made by the writer indicate that springtime budding, when stock and buds are still dormant, has possibilities.

Considerable investigation has been carried on in the Division of Forest Pathology and by other agencies on propagation of chestnut from cuttings. Greenwood cuttings are usually the most promising, but the percentage of cuttings taking root has always been low in spite of the numerous treatments used. Bretz and Swingle[1] have rooted leafbud cuttings of chestnut but high percentages of rooting have not yet been obtained. If a way were found to propagate chestnut cuttings efficiently, it would be possible to investigate compatibility of stock and scion, to compare various planting sites by the use of a single clone, and to compare clones of races or hybrids on a single site.

Summary

Chestnut breeding, to develop vigorous, blight-resistant timber trees, began in the Division of Forest Pathology, Bureau of Plant Industry, Soils, and Agricultural Engineering, U. S. Department of Agriculture in 1909 and is continuing. In the period 1925-49 more than 6,000 hybrids were grown. About 40 percent of the total possible number of combinations of crosses between the 13 species of *Castanea* have been made successfully, indicating the close relationship existing between the species.

The period of receptivity of chestnut stigmas was determined as beginning a few days after the staminate catkins began to blossom, reach-

ing its highest degree about 12 days later, and extending nearly three weeks. Pollinations made in the morning were more effective than those made in the afternoon. Attempts to obtain viable seed by self-pollinating pistillate flowers whose styles were clipped off failed; however, viable seed was obtained by cross-pollination. Tests designed to prevent insects from visiting flowering twigs on which all staminate flowers had been emasculated showed that chestnut is wind-pollinated. Exposed pollen on anthers is at first tacky, but air currents soon dry the pollen and, together with the movements of insects feeding on the pollen and nectar of the catkins, it is dislodged and is airborne.

Inheritance of blight resistance probably is controlled by two pairs of genes. Early flowering is dominant over late flowering. At Glenn Dale, Md., temperature departures from the mean for April, and occasionally for April and May, determine when flowering begins. Time of nut drop in chestnut is inherited without dominance. Time of flowering determines the time of nut drop. Size of nuts is inherited with incomplete dominance, tending to approach the nut size of the parent producing the smaller nuts. The everbearing character found in *C. seguinii* is inherited in at least a 3:1 ratio in the second generation.

First-generation hybrids from crossing a selection of *C. mollissima* with *C. dentata* appear promising for planting as forest-timber trees. On a poor site they have grown at the rate of 2.8 feet per year for 10 years. Their blight resistance is sufficient to warrant testing them on forest sites.

Scions of Japanese chestnut carrying the mutation "spineless" were grafted and later crossed with other *Castanea* species. The mutation "cracked-bark," wherein the bark of chestnut trees begins to crack at the age of three or four years, was obtained in first-generation hybrids from a *C. mollissima* × *C. dentata* cross. Stunted growth, a general drooping of the branches, and an increased susceptibility to blight and to winter injury are associated with cracked bark. An anomaly, "naked chestnut," was observed in certain hybrids; the burrs stop developing at an early stage while the nuts continue normal development, so that the green nuts are exposed. The anomaly occurs in certain years and probably is a nonheritable mutation.

Chestnuts, chinkapins and hybrids graft readily, but are difficult to bud. Extensive investigations have failed to develop an efficient method of rooting cuttings.

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